

A Special Connection between $\gamma\delta$ T Cells and Natural Antibodies?

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Abstract Natural antibodies (NAbs) play an important role in early host defense, autophagy and tissue remodeling, and in immune regulation. They arise spontaneously (without specific immunization), and are already present at birth. NAbs are produced by B1 B cells, MZ B cells and other B cell types. They include all major Ig subclasses but IgM antibodies are prevalent, especially early in development. NAbs may be poly-specific, recognize particular auto-antigens, or detect neo-determinants such as those exposed during apoptosis or generated by oxidation. NAbs do not require cognate T cell help but depend on soluble mediators produced by T cells. Our recent studies suggest that $\gamma\delta$ T cells may have a special relationship with NAbs, and play a prominent role in their regulation, in part through the fine-tuning of IL-4 levels. The spontaneously activated state of these cells likely enables their cytokine production and other functions in the absence of external stimulation. Ontogenetically, the earlier arising $\gamma\delta$ T cells are better positioned than $\alpha\beta$ T cells to shape the developing repertoire of NAbs. Intriguingly, ligand specificities of NAbs and $\gamma\delta$ T cell receptors appear to be overlapping,

perhaps allowing $\gamma\delta$ cognate help for certain NAb specificities. Via NAbs, $\gamma\delta$ T cells could exert a regulatory influence on numerous processes in health and disease.

Keywords Natural antibodies · $\gamma\delta$ T cells · Immune system development

Abbreviations

NAb Natural antibody
TCR T cell receptor
IL-4 Interleukin 4
IVIG Intravenous immunoglobulin

Introduction

Prior to any immunization, circulating antibodies already exist in normal healthy humans and mice (Avrameas 1991; Lutz et al. 2008). They are present at birth, and in mice have been shown to arise under germ-free conditions. These natural antibodies (NAbs) include all Ig subclasses. Natural IgM is prevalent particularly early in ontogeny and has been studied most extensively. B1 B cells appear to be the primary source of NAbs in mice (Savage and Baumgarth 2015), but other B cell types, most notably marginal zone B cells (Durand et al. 2009), contribute as well (Avrameas and Selmi 2013; Avrameas 2016). Although sometimes referred to as “non-specific”, NAbs in fact may be mono- or poly-specific, and recognize auto-antigens, neo-antigens and certain foreign antigens. Importantly, recent studies revealed that NAbs play a critical role in the early host defense against pathogens, protection against malignancy, tissue homeostasis and immune regulation. Available information about NAbs and treatment

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opportunities with intravenous immunoglobulin (IVIG) has been expertly reviewed by others (Ehrenstein and Notley 2010; Gronwall and Silvermann 2014; Madi et al. 2012; McCoy et al. 2006; Panda and Ding 2015; Rahyab et al. 2011; Schwartz-Albiez et al. 2009; Vas et al. 2013). It is only summarized here, followed by a discussion of new data suggesting that $\gamma\delta$ T cells become involved in regulating NAb and their functional activity.

Specificity and Function of NAb

The primary B cell repertoire is not random (Perlmutter et al. 1985). Evidence for recurrent VH gene rearrangements in mice, humans and other vertebrates, at a time when the repertoire is not yet affected by antigenic selection, has been reported (reviewed in Vas et al. 2013). Use of microarray chips allowing the simultaneous detection of antibodies specific for up to 300 defined self antigens revealed that IgM repertoires in cord blood were very similar between individuals indicating that different humans are born with the same autoantibodies produced in utero regardless of variances in IgG autoantibodies found in their mothers (Madi et al. 2009). The mechanisms responsible for this consistency are not yet fully understood.

The early IgM antibodies in mice and humans are mostly germline-encoded and are produced by CD5⁺ B1a B cells (Savage and Baumgarth 2015). They carry specificities for common bacterial antigens, auto-antigens, certain phospholipids, DNA and several cell membrane proteins. Table 1 lists some of the specificities of these NAb (Air 2015; Basnet et al. 2010; Bohn et al. 1994; Bovin 2013; Buneva et al. 2013; Chen et al. 2009; Chikazawa et al. 2013; Chou et al. 2009; Durrbach et al. 2007; Fukuda et al. 2004; Hamanova et al. 2014; Hardy and Hayakawa 2005; Kalyanaraman et al. 1982; Kulik et al. 2009; Lebon et al. 2011; Li et al. 2009; Llorente et al. 1999; Morales-Buenrostro et al. 2008; Posner et al. 1981; Robert-Guroff et al. 1982; Sauerborn et al. 2011; Shilova et al. 2011; Silvermann 2015; Skurnik et al. 2012; Toth et al. 1984; Tsiantoulas et al. 2013; Tuominen et al. 2006; Turunen et al. 2012; Wang et al. 2013; Xu et al. 2013). Other important targets of NAb include the Thomsen-Friedenreich tumor antigen (CD176) (Ulsemer et al. 2013), neural gangliosides relevant in Guillain-Barre syndrome (Boffey et al. 2004) and amyloid in Alzheimer's disease (Kayed et al. 2011). Intriguingly, many of the autoreactive specificities are similarly found throughout evolution (Flajnik and Rumfelt 2000; Gonzalez et al. 1988; Marchalonis et al. 1993). The non-random nature of the natural repertoire suggests that these early antibodies might be programmed to enable normal development, ensure essential functions and protect against common pathogens.

Indeed, natural IgM has been characterized as protective in infections with Influenza virus, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* (reviewed in Ehrenstein and Notley 2010). Natural IgM also affects B cell development. Mice genetically deficient in surface (s)IgM exhibit changes in B cell subsets including B1a B cells and MZ B cells along with increased splenic and impaired peritoneal B cell survival (Baker and Ehrenstein 2002; Notley et al. 2010). Deficiency in sIgM has also been linked to increases in autoimmunity and atherosclerosis in both humans and mouse models. Self-reactive IgE exacerbates interferon responses in autoimmunity (Henault et al. 2016). Figure 1 lists some of the known functions of NAb (Aksentijevich et al. 1991; Benatuil et al. 2005; Britschgi et al. 2009; Chen et al. 2009; Dimayuga et al. 2009; Elluru et al. 2014; Elvington et al. 2012; Frostegard 2010; Galkina and Ley 2009; Gounopoulos et al. 2007; Gronwall et al. 2012; Lobo et al. 2014; Matter and Ochsenbein 2008; McCoy et al. 2006; Nogueira-Martins and Mariano 2010; Panda et al. 2013; Pires et al. 2010; Rapaka et al. 2010; Shimomura et al. 2008; Sjoberg et al. 2009; Stager et al. 2003; Veljkovic et al. 2010; Warrington and Rodrigues 2010; Warrington and Lewis 2011; Xu et al. 2008; Zabel et al. 2013).

Influence of T Cells and Cytokines

B cells are quite capable of producing antibodies in the absence of any T cell help, including isotypes that require class switch recombination. However, in our mouse facility, mice lacking all T cells (B6.TCR- $\beta^{-/-}$ / $\delta^{-/-}$) had serum total Ig levels that reached only about 1/4 of those in wild-type mice. Levels of IgM and surprisingly, IgE antibodies were nearly normal, whereas IgA and IgG antibodies were significantly reduced, especially IgG1 (Huang et al. 2015). Cytokines produced by non-B cells can determine development and steady state levels of NAb. For example, interleukin (IL)-18 can boost production of protective natural IgM (reviewed in Ehrenstein and Notley 2010). Natural IgE is also produced in the absence of MHCII cognate help but its production depends on IL-4 (McCoy et al. 2006). Their cytokine dependence subjects NAb to the non-specific regulatory influence of other cell types.

A Connection Between $\gamma\delta$ T Cells and IgE Production in Non-Immunized Mice

Lymphocytes expressing $\gamma\delta$ T cell receptors (TCRs; $\gamma\delta$ T cells) represent a lineage whose biological role remains poorly defined. Functionally, they resemble $\alpha\beta$ T cells (Bonneville et al. 2010) but in terms of specificity and

Table 1 Broad target range of natural antibodies

Category	Antigens recognized	Species	References
Pathogen-derived	Poly- <i>N</i> -acetyl glucosamine (<i>Staphylococcus aureus</i> capsule)	Human	Skurnik et al. (2012)
	<i>Porphyromonas gingivalis</i> gingipain		Turunen et al. (2012)
	Pneumococcal virulence proteins		Lebon et al. (2011)
	Adenovirus type 5	Mouse	Xu et al. (2013)
	HIV	Human	Llorente et al. (1999)
	HTLV, simian type C virus		Toth et al. (1984)
	p24 structural core protein		Kalyanaraman et al. (1982)
	p19		Robert-Guroff et al. (1982)
	Influenza		Posner et al. (1981)
	Measles virus		Air (2015)
Autologous	Apoptotic cells including thymocytes and senescent erythrocytes and their antigens phosphorylcholine	Mouse, human	Durrbach et al. (2007)
			Chou et al. (2009)
			Silvermann (2015)
			Chikazawa et al. (2013)
			Hardy and Hayakawa (2005)
			Chen et al. (2009)
	Tumor cells, activated T cells	Human	Bohn et al. (1994)
	Proliferation-related self peptides		Fukuda et al. (2004)
	<i>Annexin IV</i>	<i>Mouse, human</i>	<i>Kulik et al. (2009)</i>
	<i>Nucleic acids</i>	<i>Human</i>	<i>Buneva et al. (2013)</i>
Allogeneic	Glycans, sialoglycans	Chicken, human	<i>Henault et al. (2016)</i>
			Shilova et al. (2011)
			Bovin (2013)
	Bone morphogenic protein	Human	Sauerborn et al. (2011)
	IFN- β , IFN- γ		Sauerborn et al. (2011)
	$\alpha(1,3)$ -galactosyl		Hamanova et al. (2014)
	CD40		Elluru et al. (2014)
	HLA		Morales-Buenrostro et al. (2008)
	Xenogenic		Li et al. (2009)
	<i>N</i> -glycolylneuraminic acid	Mouse	Basnet et al. (2010)
Oxidation-induced	Apoptotic cells	Mouse, human	Chou et al. (2009)
	Oxidized lipids, cardiolipin	Mouse	Tsiantoulas et al. (2013)
			Tuominen et al. (2006)
	Malondialdehyde acetaldehyde adducts	Human	Wang et al. (2013)

Reference numbers in the table refer to list of references following the main body of the text. Marked in italics: NABs with pathogenic effect

mechanism of TCR-ligand recognition, they share properties with B cells (Chien et al. 1996; Zeng et al. 2012). In several studies with mice carrying distinct mutations affecting TCR-signaling, increased non-immune IgE levels could be correlated with increases in $\gamma\delta$ T cells (Nunez-Cruz et al. 2003). In particular, this was observed in mice deficient in the Tec family tyrosine kinase Itk, which are unable to mount conventional IL-4 or IL-4 plus IL-13 producing T helper 2 (Th2) responses (Felices et al. 2009; Qi et al. 2009). Nevertheless, such mice had elevated levels of serum IgE and increased numbers of germinal center B cells, a phenotype found to be dependent on the presence of

$\gamma\delta$ T cells. $\gamma\delta$ T cells in *Itk*^{-/-} mice produced more Th2 cytokines and expressed at elevated levels co-stimulatory molecules important for B cell help, which suggested that they directly promote B cell activation and Ig class switching. In the same year, data generated in our lab with TCR-signaling competent mice revealed that IgE levels (both pre- and post-immunization) are determined by the composition of $\gamma\delta$ T cells. Although mice deficient in all $\gamma\delta$ T cells had slightly reduced levels of serum IgE, others lacking only V γ 4⁺ and V γ 6⁺ subsets had vastly increased background IgE levels as well as exacerbated IgE responses following immunization (Huang et al. 2009). In these

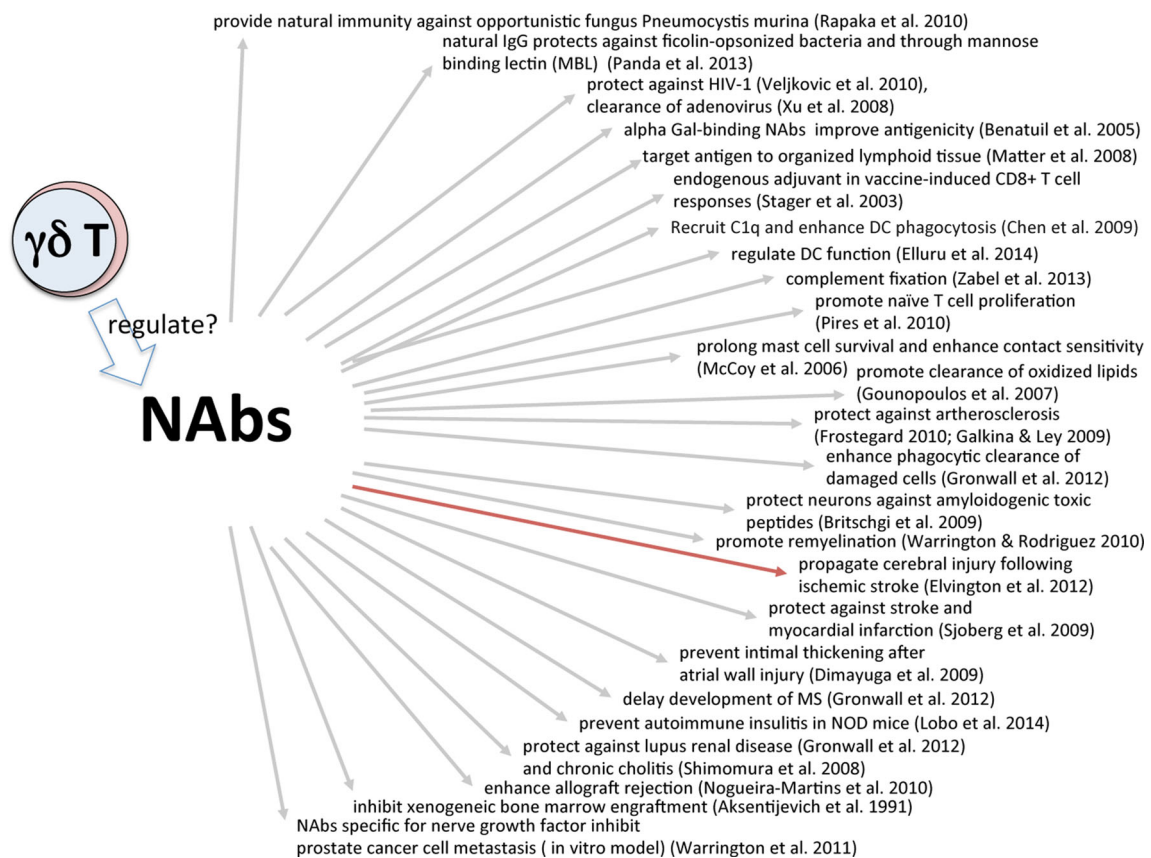


Fig. 1 Diverse functions of natural antibodies. Marked in red: NAb with pathogenic effect

mice, $V\gamma 1^{+}$ and especially the NKT-like IL-4-producing $V\gamma 1/V\delta 6.3^{+}$ $\gamma\delta$ T cells (Gerber et al. 1999) were much increased, and so were serum levels of IL-4 (Huang et al. 2015). In fact, the genetically $\gamma\delta$ -deficient mice (B6.TCR- $V\gamma 4/6^{-/-}$) closely resembled $Itk^{-/-}$ mice. Together, these observations implied that the composition of $\gamma\delta$ T cells can play a critical role in setting steady state levels of IgE antibodies in non-immunized mice.

Effect of $\gamma\delta$ T Cells on Serum Ig Levels in Non-Immunized Mice

After these findings, we extended the analysis to other Ig classes (Huang et al. 2015). Our data suggest that the net effect of $\gamma\delta$ T cells on total serum Ig in normal non-immunized C57BL/6 mice is slightly enhancing as adult animals genetically deficient in all $\gamma\delta$ T cells (B6.TCR- $\delta^{-/-}$) had approximately twofold lower Ig levels. Net total Ig levels in these mice are lower mainly due to significantly reduced levels of IgG1 and IgG2b. Further insight came from the analysis of partially $\gamma\delta$ T cell-deficient mouse strains. Thus, mice lacking $V\gamma 1^{+}$ $\gamma\delta$ T cells exhibit total Ig levels that are even further diminished than those in

B6.TCR- $\delta^{-/-}$ mice, and show much reduced levels of IgM, IgG3, IgG1 and IgG2b. IgG2c and IgA were diminished also but to a lesser degree. Hence, $V\gamma 1^{+}$ $\gamma\delta$ T cells appear to enhance the production of several Ig subclasses in non-immunized mice. On the other hand, mice deficient in $V\gamma 4^{+}$ and $V\gamma 6^{+}$ $\gamma\delta$ T cells exhibit much increased total Ig levels, mainly due to increases in IgM, IgG1, IgG2b and IgG2c. IgE antibodies are even more elevated but, due to their low concentration, contribute only little to overall increases in Ig levels. IgG3 and IgA are not affected. Hence, $V\gamma 4^{+}$ and perhaps $V\gamma 6^{+}$ $\gamma\delta$ T cells, two subsets in mice distinguished by their ability to produce IL-17 (O'Brien et al. 2009), appear to exert an inhibitory effect on most Ig production under steady state conditions. Our data suggest that this inhibition in non-immunized mice is mediated in large part through regulating $V\gamma 1^{+}$ $\gamma\delta$ T cells, their IL-4 production, and IL-4-dependent IL-4 production by $\alpha\beta$ T cells (Huang et al. 2015). Consistently, for some Ig subclasses, most obviously IgM, but also IgG2b and IgG2c, this regulation is still evident even in the absence of $\alpha\beta$ T cells. Finally, it has been shown that $V\gamma 1^{+}$ $\gamma\delta$ T cells kill tissue macrophages and thus resolve inflammatory immune responses (Carding and Egan 2000; Dalton et al. 2003). As macrophages can be a source of IL-18, this cytotoxic

activity might enable indirect control of IL-18-dependent antibody-types, at least in inflammation.

Comparing serum Ig levels in non-immunized adult wt C57BL/6 mice with mice lacking all $\gamma\delta$ T cells (B6.TCR- $\delta^{-/-}$) or all $\alpha\beta$ T cells (B6.TCR- $\beta^{-/-}$) initially showed that the absence of $\alpha\beta$ T cells affects levels of total serum Ig and most Ig subclasses more than does the absence of $\gamma\delta$ T cells. IgE antibodies in non-immunized mice were a notable exception: They were diminished in the absence of all $\gamma\delta$ T cells but not in the absence of all $\alpha\beta$ T cells (Huang et al. 2015). However, mice lacking only subsets of $\gamma\delta$ T cells revealed far greater changes in Ig levels, suggestive of potent enhancing and suppressive regulation by these cells (Huang et al. 2015). This powerful regulation is all the more noteworthy considering that $\gamma\delta$ T cells in circulation and in the lymphoid tissues represent a much smaller cell population than $\alpha\beta$ T cells.

$\gamma\delta$ T Cells Affect the Repertoire of Antibodies in Non-Immunized Mice

The observation that changes in $\gamma\delta$ T cells differentially affect levels of Ig subclasses in non-immunized mice suggests an influence on the repertoire and specificities of NAbs. To explore this possibility, we examined antibodies specific for chromatin, DNA and nuclear antigens in non-immunized mice (Huang et al. 2015). We found that the same change in $\gamma\delta$ T cells that increased all Ig subclasses except IgG3 and IgA—a genetic deficiency in $V\gamma 4^+$ and $V\gamma 6^+$ $\gamma\delta$ T cells, also led to substantial increases in anti-chromatin antibodies. However, the anti-chromatin antibodies were still further increased relative to the elevated total Ig levels, indicating a change in the composition of the elevated antibodies, and in their specificity. We also found a clear increase in antibodies detecting dsDNA/histone complexes and smaller increases in antibodies specific for ssDNA, and anti-nuclear autoantibodies. Finally, cell transfer and depletion experiments showed the change in antibody composition/specificity to be driven by the dys-regulated $V\gamma 1^+$ $\gamma\delta$ T cells derived from non-immunized B6.TCR- $V\gamma 4/6^{-/-}$ mice. Hence, $\gamma\delta$ T cells affect the antibody repertoire in non-immunized mice (Huang et al. 2015).

Do $\gamma\delta$ T Cells Have a Special Connection with NAbs?

The above-mentioned observations clearly implicate $\gamma\delta$ T cells in the regulation of antibodies independently of immunization, and thus establish their involvement with

NAbs. However, it remains to be determined if $\gamma\delta$ T cells have a unique functional connection with NAbs. Do they play a role distinct from that of other T cells, in the development and maintenance of these antibodies? Such a role might even help to explain the evolutionary conservation of $\gamma\delta$ T cells. We suggest that such a special connection is probable, for the following reasons: Firstly, unlike the majority of unstimulated $\alpha\beta$ T cells, which are antigenically naïve and metabolically inactive, many peripheral $\gamma\delta$ T cells in non-immunized mice already exist in a state of moderate activation (Tough and Sprent 1998). This activated/memory phenotype could support background levels of cytokine production capable of sustaining some B cell differentiation and spontaneous antibody production. Secondly, in mouse ontogeny, $\gamma\delta$ T cells develop roughly at the same time as the earliest B cells, and for a short period of time, the fetal liver gives rise to precursors of both cell types (Havran and Allison, 1988; Whitlock et al. 1985). $\alpha\beta$ T helper cells, on the other hand, arise several days later (Marrack et al. 1988). Moreover, $\gamma\delta$ thymus-emigrants are functionally more mature than $\alpha\beta$ thymus-emigrants (Jin et al. 2009; Narayan et al. 2012). In particular, $\gamma\delta$ thymocytes are already sufficiently differentiated to produce polarized cytokines including IL-4 and IL-13 (Jin et al. 2009). Thus, $\gamma\delta$ T cells are enabled to support and modulate B cell development and antibody production from the start, by supplying cytokines prior to any immunization. In addition, $\gamma\delta$ T cells might interact with B cells via ligands expressed on B cells such as members of the CD1 family of molecules (Delia et al. 1988; Duan et al. 2008; Sonoda and Stein-Streilein 2002), which are recognized by $\gamma\delta$ T cells (Dieude et al. 2011; Porcelli et al. 1992; Russano et al. 2006). Lastly, one might consider the early idea that B1 B cells and $\gamma\delta$ T cells occupy equivalent positions in a “layered immune system” (Herzenberg and Herzenberg 1989). We actually suspect that there is resemblance or overlap between antigen specificities of NAbs and $\gamma\delta$ T cells. Thus, both recognize poly-anionic ligands including certain synthetic polypeptides (Cady et al. 2000) but also DNA/protein (unpublished data) and phospholipid/protein complexes (Born et al. 2003; Dieude et al. 2011; Lafer et al. 1981), and recent studies suggest a shared specificity for insulin (Aydintug et al. 2014; Liu et al. 2002; Zhang et al. 2010). Thus, in addition to non-specific cytokine support, at least some $\gamma\delta$ T cells conceivably could provide help to B cells via cognate interactions between $\gamma\delta$ TCR, antigen and B cell receptor. Hence, the extent to which antigen specificities of $\gamma\delta$ TCRs and NAbs might be related in non-immunized mice would be revealing with regard to the biological role of $\gamma\delta$ T cells.

Modulating NAbS and Their Contribution to Immune Responsiveness via $\gamma\delta$ T Cells?

Although B cells can spontaneously produce antibodies in the absence of all T cells, T cells affect antibody production when they are present. The assumption that $\gamma\delta$ T cells modulate levels and repertoire of NAbS invites additional points: First, $\gamma\delta$ T cells in humans undergo large changes in population size and composition during ontogeny (Parker et al. 1990), due to genetic variation, in the course of hematopoietic transplantation (Airoidi et al. 2015), and as a consequence of diseases (Bank and Marcu-Malina 2013; Pauza et al. 2015). Thus, one might expect based on the findings in mice that such changes in human $\gamma\delta$ T cells have an effect on human B cells and their natural production of immunoglobulins. Large variation of NAbS might occur as a consequence of the changes in human $\gamma\delta$ T cells. Furthermore, similar to the mice, specific targeting of the relevant human $\gamma\delta$ T cells, directly or through their regulators, might effectively change human NAbS, complement IVIG in certain cases, and at times might be preferable over targeting all B cells (e.g., using antibody drugs against CD19) or entire antibody classes (e.g., antibody drugs against IgE).

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