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The Innate Biologies of Adaptive Antigen Receptors

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Abstract

Nonclonal innate immune responses mediated by germ line–encoded receptors, such as Toll-like receptors or natural killer receptors, are commonly contrasted with diverse, clonotypic adaptive responses of lymphocyte antigen receptors generated by somatic recombination. However, the Variable (V) regions of antigen receptors include germ line–encoded motifs unaltered by somatic recombination, and theoretically available to mediate nonclonal, innate responses, that are independent of or largely override clonotypic responses. Recent evidence demonstrates that such responses exist, underpinning the associations of particular γδ T cell receptors (TCRs) with specific anatomical sites. Thus, TCRγδ can make innate and adaptive responses with distinct functional outcomes. Given that αβ T cells and B cells can also make nonclonal responses, we consider that innate responses of antigen receptor V-regions may be more widespread, for example, inducing states of preparedness from which adaptive clones are better selected. We likewise consider that potent, nonclonal T cell responses to microbial superantigens may reflect subversion of physiologic innate responses of TCRα/β chains.

INTRODUCTION

For jawed vertebrates, adaptive immunity is among the major sources of immune variation, within any one individual over time and between individuals. It reflects dynamic, clonally restricted responses of lymphocytes to diverse antigens, responses that in turn are facilitated by immensely diverse lymphocyte antigen receptors: immunoglobulin (Ig), T cell receptor (TCR) αβ, and TCRγδ.

As considered for Ig by Tonegawa (1), the root source of antigen receptor diversity lies in the assembly of heterodimers that compose each type of antigen receptor: Ig light (L) and heavy (H) chains, TCRα and β chains, and TCRγ and δ chains. Because the genes for each chain compose multigene families, the first contributor to diversity is quasirandom chain pairing: e.g., any one of many IgL chains can be paired with any one of many IgH chains, and likewise for TCRα and TCRβ and for TCRγ and TCRδ. The second source of diversity is combinatorial and reflects the fact that the Variable (V) region of each chain is assembled following the quasirandom, recombinase-activating gene (RAG)1- and RAG2-dependent somatic recombination of noncontiguous germ line gene segments: Variable (V), Diversity (D), and Junction (J) segments for IgH, TCRβ, and TCRδ chains and V and J segments for IgL, TCRα, and TCRγ chains. The third source of variation is junctional, in that different amino acid sequences result from the imprecision of RAG1/2-dependent somatic recombination, with variable nucleotide loss and addition. Fourth, additional variation is introduced into rearranged Ig-coding sequences by AID-dependent somatic mutation (2). Although this may also occur in TCRs in nurse sharks and some other species (3), the general rule is that TCRs are unmutated.

The relevance of these diversifiers to antigen specificity relates to structural topology. The sequences that are most variable among inherited V-gene segments for Igs and TCRs are clustered within short, hypervariable (HV) regions 1 and 2 that are noncontiguous in the DNA sequences, but which encode protein residues that cluster together on the antigen receptor surface (4). Those residues are spatially integrated with those encoded by variable junctional regions; hence, three HV regions from each chain together compose the complementarity-determining region (CDR) that directly binds antigen. HV1 and HV2 contribute to CDR1 and CDR2, respectively, while the sequences encoded by the variable junctional region compose CDR3.

Understandably, therefore, most consideration of antigen receptor specificity in relation to adaptive immunity has focused on the CDR, and particularly the contributions of CDR3, which is a direct product of somatic imprecise gene rearrangement, and which can be immensely diverse, offering vertebrates the capacity to make highly specific responses to any one of myriad challenges. By contrast, HV1 and HV2 sequences are inherited and identical in all antigen receptors that use the same V-gene segment, notwithstanding their potential to be somatically mutated in Ig. The significance of HV1 and HV2 is illustrated by their critical contributions to microbial/viral antigen recognition by antibodies, particularly in the early stages of responses (5), and by immunodeficiencies that may be associated with V-gene segment polymorphisms. Nonetheless, the conformational integration of CDR1 and CDR2 with CDR3 means de facto that their contributions to antigen specificities are most often clonally restricted. Hence, there has until recently been less focus on how antigen receptor biology may be influenced purely by inherited variation.

This review seeks to reevaluate this perspective. In particular, HV4 is an additional region of inherited sequence variation that encodes residues that topologically border CDR2 but that are displaced relative to the clonotypic CDR (**Figure 1**). Thus, any interactions mediated by CDR2+HV4 should constitute a nonclonally restricted property of all antigen receptors expressing that germ line V-gene segment motif. A nonclonally restricted system of recognition is a defining signature of innate immunity (6). While T and B cells both display innate responsiveness, e.g., mediated by TLRs, costimulators, cytokines, or natural killer (NK) cell receptors, and whereas the

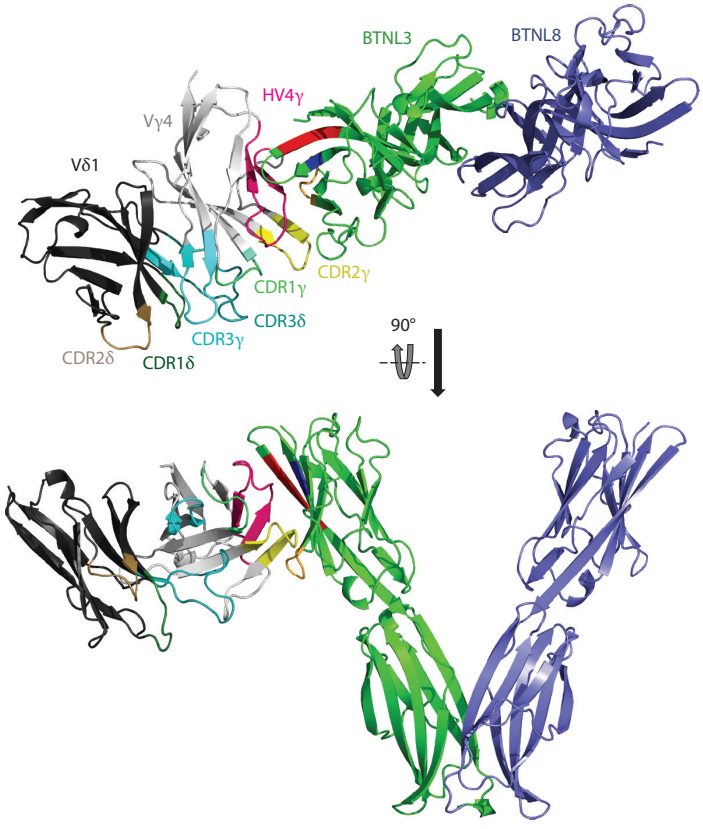


Figure 1

Mode of interaction between a Vγ4Vδ1 T cell receptor and BTNL3+8. Cartoon representation (PyMOL) of the result of molecular docking using SwarmDock. CDR1,2,3γ/δ and HV4γ are highlighted and annotated. Motifs mapping to the CFG face of BTNL3 that were shown to make critical contributions to the interaction are highlighted in orange, blue, and red. Adapted from Reference 21.

constant regions of antigen receptors enter into innate interactions, e.g., with F_c receptors, this review focuses on the potential for antigen receptor V-regions to mediate innate responses.

Conceivably, such innate recognition potentials of antigen receptor V-regions might explain the disproportionate use of IgV gene segments in particular settings, such as autoreactive B cell lymphomas. It can likewise contribute to the pathogenic, nonclonal targeting of whole swathes of the αβ T cell compartment by Vβ-specific or Vα-specific superantigens. Nonetheless, the relevance of either of these scenarios to the normal physiologic development and/or function of lymphocytes has been uncertain, and hence it has been difficult to link them to inheritance patterns for particular V-gene segments. By contrast, recent data have revealed an evolutionarily conserved innate mechanism by which essentially all γδ T cells expressing a specific Vγ-gene segment are positively selected by an intestine-specific endogenous ligand, without which mechanism the normal intestinal T cell repertoire does not form.

This emerging biology of γδ T cell regulation strongly suggests that innate recognition by antigen receptor V-regions elicits responses, such as a tonic state of preparedness, that are distinct from those responses, such as full effector differentiation, that are elicited by engagement of clonally restricted antigens. The potential to make qualitatively distinct innate-like and adaptive

responses, respectively, argues that individual antigen receptors may have "adaptate" capabilities that may contribute to critical, albeit poorly understood, aspects of lymphocyte biology.

BTNL PROTEINS REGULATE γδ T CELLS

Human butyrophilin-like (BTNL) and mouse Btnl type I transmembrane proteins are encoded by small multigene families seemingly conserved in placental mammals, and possibly beyond. They are members of the Ig superfamily (SF) and mostly display a membrane-distal IgV domain N-terminal to a membrane-proximal IgC domain that is connected via a transmembrane domain to various types of intracellular C-terminal domains. They show clear sequence relatedness to the B7-SF of lymphocyte regulators that includes CD80, CD86, ICOS-L, and PD-L1 and PD-L2 (7). The mouse *Btnl* genes include the *Skint* subfamily, whose prototypic member, *Skint1*, encodes a V-domain for which nuclear magnetic resonance (NMR) analysis revealed striking structural similarity to the ectodomain of PD-L1 (8). Moreover, there are experiments that seemingly support a role for some Btnl/BTNL proteins in B7-like T cell regulation (9–11).

A link of Btnl proteins to γδ T cell biology was made when fetal thymic epithelial expression of *Skint1*, which was hitherto an unknown gene, was shown to be critical for the selective expansion and maturation of the progenitors of murine dendritic epidermal T cells (DETCs), which mostly express a canonical Vγ5Vδ1 TCR (12–14). Clearly, the association might have been driven by a hitherto uncharacterized receptor expressed exclusively by DETC progenitors. Alternatively, it might have been driven by the TCR. Were this to be the case, it would not have been immediately apparent that a specific, germ line–encoded subregion of the TCR might drive *Skint1* dependence, since canonical DETC progenitors express identical Vγ5 and Vδ1 chains with identical CDR3s, in aggregate recognized by a clonotypic antibody, 17D1 (15), and the primary phenotype of *Skint1* mutant mice is loss of 17D1$^+$ DETCs (14). This notwithstanding, the Vγ5-gene segment emerged as a strong candidate when it became clear that *Skint1* does not regulate the thymic progenitors of uterine T cells, which express a canonical Vγ6Vδ1 TCR that is largely identical to the DETC TCR, including in CDR3, but which uses Vγ6 versus Vγ5 (14).

Following this, it was unexpectedly discovered that a well-established, proliferative effector response of human peripheral blood Vγ9Vδ2 T cells to low-molecular-mass organic phosphates, specifically hydroxymethylbut-2-enyl pyrophosphate (HMBPP) and isopentenyl pyrophosphate (IPP) (so-called phosphoantigens), depended on butyrophilin (BTN) 3A1 expression by the phosphoantigen-sensitized cell (16). Unlike DETCs, phosphoantigen-reactive γδ T cells do not all express a canonical TCR, yet the requirements for response are primarily the Vγ9 chain, with few very strict requirements on CDR3 length or sequence (see below). Recent studies have suggested that cells expressing such TCRs are positively selected in the human fetal thymus, possibly by a BTN3A1-dependent process (17). Because of its mandatory association with Vγ9, the phosphoantigen response has been widely considered to mimic the nonclonal responses to superantigens of αβ T cells expressing defined Vβ-gene segments, although there is as yet no experimental evidence for this, a point considered at greater length below.

The next association of Btnl proteins with γδ T cell regulation was identified when the selective proliferation and maturation of murine intestinal epithelial lymphocytes (IELs) expressing the signature Vγ7 chain were found to be severely impaired in *Btnl1* knockout mice (18). *Btnl1* is expressed by enterocytes, but while necessary it was not sufficient for eliciting Vγ7$^+$ IEL responses, requiring instead that it be coexpressed with *Btnl6*. Displaying unanticipated evolutionary conservation, a large subset of human colonic IELs expressing the Vγ4 chain could be specifically activated by cells transduced to express *BTNL3* and *BTNL8*, two genes ordinarily transcribed by human gut epithelial cells (18).

Shortly thereafter, it was shown that while necessary, BTN3A1 was also not sufficient for inducing optimal phosphoantigen responses of Vγ9Vδ2 T cells, requiring instead its coexpression with BTN3A2 (19) and with BTN2A1 (20). The cell surface expression of BTN3A1 was regulated by BTN3A2, and the two proteins could be coimmunoprecipitated, consistent with their entering into IgC domain–dependent heterodimers. Similar behavior was shown for murine Btnl1 and Btnl6, and for human BTNL3 and BTNL8 proteins, suggesting that BTN/BTNL/Btnl heterodimers compose the active biological entities that regulate the developmental maturation and/or activation of specific subsets of γδ T cells defined by their Vγ chain usage (18–21). Moreover, those heterodimers may form the basis for larger heterocomplexes involving three or more qualitatively distinct proteins (19, 20).

BTNL PROTEINS ARE INNATE γδ TCR LIGANDS

Despite the strong associations of Btnl/BTNL proteins with specific TCRs, it remained possible that their effects were mediated by dedicated receptors for Btnl/BTNL proteins, just as there are receptors, e.g., CD28, PD1, and ICOS, for other B7-SF proteins, specifically, CD80 and CD86, PDL1 and PDL2, and ICOSL, respectively. While not discounting that such receptors may exist, we note that none has clearly been described on γδ T cells. By contrast, the direct involvement of the TCR was suggested by the capacity of cells transduced with BTNL3+8 or Btnl1+6 to drive TCR downregulation by primary IELs of human and mouse origins, respectively (18).

TCR downregulation and CD25 upregulation, which was also observed, are common consequences of TCR engagement on conventional αβ T cells. Furthermore, the transduction of human Jurkat-derived T cell lines with human Vγ4$^+$ TCRs or mouse Vγ7$^+$ TCRs single-cell cloned from primary IELs was sufficient to confer responsiveness in *trans* to cells expressing either BTNL3+8 or Btnl1+6. By cross comparing different TCRs, it was concluded that BTNL3+8 reactivity required Vγ4, and that Btnl1+6 reactivity required Vγ7, with no strict limitations on the TCRδ chain in either case (21).

The means by which either human Vγ4 or mouse Vγ7 chains mediated BTNL/Btnl responses was determined by mutagenesis and by constructing chimaeras with related Vγ-gene segments (21). In both cases, the critical residues responsible mapped unexpectedly to HV4, within FR3, with additional contributions of residues in CDR2γ. These regions combine to form a solvent-exposed surface that lies to the side of the aggregate CDR1–3γ/δ antigen-combining surface (**Figure 1**). Via a combination of molecular modelling and mutagenesis, the BTNL/Btnl residues critical for the interaction were mapped to noncontiguous sequences that combine in tertiary conformation to sit within a solvent-exposed CFG face of the kind used to mediate heterotypic interactions of other Ig-SF proteins, e.g., VCAM, MadCAM, CD2, and CD4 (22–25) (**Figure 1**). In each case, one of the BTNL or Btnl chains, BTNL3 or Btnl6, respectively, mediated the interaction with the TCR, while the other chains, BTNL8 or Btnl1, respectively, seemed to act primarily as chaperones, consistent with the heterodimeric forms being required for appropriate subcellular localization and/or trafficking (19, 21).

Of note, the proposed mode of interaction is completely conserved from mouse to human. Moreover, the direct and specific binding of human Vγ4 to BTNL3 has been confirmed by a combination of surface plasmon resonance and isothermal calorimetry. Depending on the method and conditions used, the affinity was 5–25 μM (26). Importantly, the binding face on Vγ4 is entirely germ line–encoded, theoretically permitting the nonclonal response to BTNL3 of essentially all Vγ4-expressing T cells. It is possible that the strength of the response varies among Vγ4$^+$ TCRs, e.g., depending on the TCRδ chain, just as different γδ TCRs show different response strengths to anti-CD3 antibodies despite neither the TCRγ nor the TCRδ chains being directly involved

in binding to those antibodies (21). Indeed, it was claimed that there was no BTNL response of a Vγ4$^+$ TCR in which the TCRδ chain displayed a particularly long CDR3δ (27), although this finding seems inconsistent with other data (26), possibly reflecting suboptimal sensitivity of the response assay employed.

Such qualifications notwithstanding, the capacity of most (and possibly all) Vγ4$^+$ TCRs to mediate responses to BTNL3+8 evokes the nonclonality that is a defining hallmark of innate immunity, as illustrated by TLR-mediated engagement of microbial molecular patterns (6). Such responses are practically and conceptually distinct from adaptive responses mediated by clonally restricted CDR1–3 surfaces that reflect combinatorial, junctional, and chain-pairing diversity. Strikingly, however, there are several Vγ4$^+$ TCRs for which clonally restricted specificities have also been identified, for example, toward CD1-lipid complexes (21, 28, 29) and toward the CD1-related molecule EPCR (30). Likewise, a small subset of murine Vγ7$^+$ cells harboring an appropriate CDR3δ germ line sequence displayed responses to T10/T22 (21), which are non-peptide-binding MHC class I (MHC-I)-related molecules. Studies that so far are limited to recognition by a single human Vγ4Vδ5 TCR of BTNL3+8 and of EPCR have shown that although the antigen-binding modalities are spatially separate, the TCR cannot bind both ligands at once, owing perhaps to steric hindrance and/or conformational change (26). Apparently, there is also no contingency of one binding mode on the other.

ADAPTATE γδ TCRs

In sum, murine Vγ7$^+$ and human Vγ4$^+$ TCRs have the potential to deploy separable innate and adaptive binding modes, seemingly to fulfill different functions of evident physiologic importance. This has been referred to as adaptate biology (31). Within this adaptate biology, the adaptive mode may facilitate responses to altered conditions in the gut epithelium that would be reflected in the upregulation of CD1-lipid complexes and of MHC-1-related molecules, such as EPCR. In fact, the universe of adaptive specificities may extend far beyond MHC-I-related proteins and CD1, particularly given the breadth of CDR3δ sequence composition (32), and multiple examples have been reported of adaptive clonal expansions and functional differentiation of γδ T cells in human blood and tissues (33–35), driven by as yet unelucidated antigens.

By contrast, the innate mode of adaptate biology is critical to mediate the selective developmental expansion and maturation of mouse intestinal Vγ7$^+$ cells (18). Moreover, given that *Btnl1* and *Btnl6* continue to be expressed beyond the developmental window in early life during which the Vγ7$^+$ IEL compartment forms, the innate mode may induce additional nonclonal responses, e.g., to prevent the cells' precocious activation while maintaining the cells' differentiated state and preparedness to respond, possibly akin to tonic signaling. An analogous function may likewise be performed by human intestinal BTNL molecules. In that regard, BTNL3+8 expression and Btnl1+6 expression are characteristics of differentiated epithelium (18, 27), thus transmitting to Vγ4$^+$ and Vγ7$^+$ IELs, respectively, both the T cells' location (i.e., the gut epithelium) and the ambient status of the tissue (i.e., steady state). In the same vein, the DETC TCR in the steady-state murine epidermis is constitutively engaged by suprabasal keratinocytes, as manifest in discrete physical complexes that upon keratinocyte dysregulation disseminate, thereby freeing DETCs to undergo full activation in response to stimuli that have yet to be molecularly defined (36).

Such scenarios evoke the functions of B7 proteins to which Btnl/BTNL proteins are evolutionarily most related: namely, the contextual communication of the status of the local milieu, thereby to license or otherwise regulate lymphocyte responses to antigen. However, in the adaptate scenario, both the contextual information and the antigenic stimulus would be received via a single molecule, the γδ TCR. Currently, this scenario is wholly hypothetical. Available

biochemical and cellular data argue that the innate and adaptive modes of TCRγδ are separable, and there is as yet no experimental evidence that a single TCR responds to innate and to adaptive stimuli, presumably sequentially. Instead, some T cells expressing a human/mouse Vγ4/Vγ7 TCR might at steady state be regulated by the innate mode, and upon tissue dysregulation respond rapidly to stress-regulated molecules, such as MICA/Rae-1, by use of innate receptors, such as NKG2D, rather than the TCR (37–39). This might in turn link sequentially to the adaptive biology of other lymphocytes. For example, whereas the innate activation of myeloid cells is commonly regarded as a prerequisite for antigen-specific αβ T cell responses, lymphoid stress surveillance refers to an immunogenic state that is initiated by the rapid, nonclonal activation of lymphocytes, including tissue-associated γδ T cells (37).

Adaptive γδ T cells, conversely, might be cells that were never previously subject to innate regulation via the TCR. Between these extremes lie other possibilities, for example, spatially colocalized TCRs might use the innate mode, whereas other TCRs on the same cell might employ the adaptive mode: Moreover, the two classes of ligand might be engaged on different cells or different cell types, e.g., an epithelial cell and a myeloid cell.

THE GENERALITY OF INNATE γδ TCRs

One key question to address is the generality of innate γδ TCR recognition. Clearly, as yet unidentified molecules unrelated to BTNL and Btnl proteins might interact innately with other Vγ chains in humans and mice, respectively. Within the BTNL/Btnl family, innate γδ TCR regulation may underpin the regulation of DETCs by *Skint1*. Nonetheless, although *Skint* selection is responsible for the selective maturation of Vγ5$^+$ DETCs, the primary defect in *Skint1*-mutant mice, as considered above, is the ablation of DETCs coexpressing Vγ5 and a Vδ1 chain with a canonical CDR3 (13–15). Given this, it is possible that *Skint*-dependent DETC selection represents an aggregate of contributions from the Vγ5 chain, the Vδ1 chain, and CDR3 and is therefore not a nonclonal property of essentially all Vγ5-expressing γδ T cells.

Provocatively, the phosphoantigen response of human peripheral blood Vγ9Vδ2 T cells was ablated by mutating HV4 residues in Vγ9 that are counterparts to those in Vγ4 that engage BTNL3 (20, 26). Likewise, the response was vulnerable to mutation of BTN3A1 and/or BTN3A2 at sites equivalent to those in BTNL3 that mediate Vγ4 interactions. Thus, this response, too, may reflect the innate interaction of germ line–encoded TCR sequences with the CFG face of BTN protein(s), specifically BTN3A1, BTN3A2, or BTN2A1. Nonetheless, as was the case for the Vγ5Vδ1 DETC TCR, BTN3A-dependent activation of the Vγ9Vδ2 TCR may involve additional regions of the TCR beyond HV4-CDR2γ. For example, whereas phosphoantigen reactivity does not depend on unique CDR3 motifs, CDR3 sequence diversity in phosphoantigen-reactive TCRs is limited, with reactivity precluded by some CDR3δ and CDR3γ sequences yet seemingly dependent on the JγP (a.k.a. Jγ1.2) junction gene segment and upon the pairing of Vγ9 to Vδ2 (40, 41).

Other durable γδ T cell responses to infection also fail to phenocopy clonal immunity (31). Such quasi-adaptive responses provided secondary protection against pathogens and included expansions en masse of murine Vγ1Vδ6.3$^+$ cells in response to malaria (42), and of Vγ6Vδ1$^+$ cells in response to intestinal *Listeria monocytogenes* infection or to repeated airway infection with *Bacillus subtilis* (43, 44). Although the cells' diversity was in each case very limited, the precise roles of the TCR in driving these responses were not determined, and so it is premature to conclude that these may reflect CDR3-independent innate responses.

Another question to address is the signal transduction pathway(s) activated by innate TCR engagement. Although the *Btnl1*-dependent development of murine Vγ7$^+$ IEL progenitors involves

selective cell expansion and phenotypic differentiation, there is little activation of effector function (18), thereby distinguishing it from conventional T cell activation. Moreover, when *Btnl1*-deficient mice were rescued by the inducible expression of a *Btnl1* transgene, expansion and differentiation were driven only during an early-life developmental window for IELs, whereas after that time point only differentiation was induced (18). This suggests that innate TCR engagement might induce different signals depending on the state of the responding T cell and/or other contextual factors, as is the case for signaling subsequent to conventional αβ TCR engagement. In addressing these and other questions, it is appropriate to consider what may be learned from the innate biologies of other antigen receptors. Reciprocally, the recent findings concerning γδ TCR biology may offer new insights into the complex biologies of TCRαβ and Ig.

SUPERANTIGENS AND INNATE TCRαβ BIOLOGY

Relative to TCRγδ, there is much more detailed understanding of CDR-mediated adaptive recognition of antigens by TCRαβ, including for unconventional αβ T cells such as CD1d-restricted type I and type II NKT cells (45), group I CD1-restricted T cells (46), and MR1-restricted MAIT cells (47). Germ line–encoded CDR1 and CDR2 regions can make critical contributions to adaptive recognition, including to the binding of MHC and MHC-related antigen-presenting molecules, but evidence for any direct involvement of HV4 is scant. Indeed, one example of HV4β affecting the affinity of rat and mouse invariant NKT (iNKT) TCRs for CD1d reflected an indirect influence (48). This is not surprising, given that HV4 lies outside of the constrained size and geometry of TCRαβ CDRs imposed by the requirement to recognize antigen in the context of MHC or MHC-related molecules, such as CD1 and MR1. Indeed, there is only limited variation in TCRαβ CDR3 lengths relative to those of Ig or TCRγδ (32).

Instead, the HV4-CDR2β regions of mouse and human αβ TCRs are commonly targeted by superantigens (**Figure 2**), which are encoded by different types of bacteria and by retroviruses. Additionally, superantigen targeting of HV4-CDR2 residues of human TCR Vα has been reported, and may be more widespread than previously thought (49). Superantigens exert profound effects on T cells ranging from unspecific hyperactivation through anergy to deletion, possibly reflecting an efficient means of microbial immune evasion that either dampens the responsiveness of microbe-specific T cells or skews the response away from clonal dominance by αβ T cells, which might otherwise focus on specific peptides whose targeting would diminish microbial fitness. By engaging germ line–encoded sequences, microbial superantigens can nonclonally target swathes of the αβ T cell compartment: In sum, they exploit the innate potentials of αβ TCRs. Nonetheless, superantigens are heterogeneous, varying in the scope and scale of their interactions with TCRβ and TCRα, and additionally interacting with MHC class II (MHC-II) molecules juxtaposed to the surface composed by CDRs1–3 (see below).

ENDOGENOUS Vβ-SPECIFIC SUPERANTIGENS

Prototypic superantigenic activity emerged from observations over 40 years ago that T cells from specific inbred mouse strains showed strong activation responses to cells of other inbred strains, despite being MHC matched (50). The unidentified drivers of this response were termed minor lymphocyte stimulating (Mls) antigens (51), the prototypic example of which was later identified as a short gene located within the 3′ long terminal repeat of integrated MMTV proviruses found in *Mlsa* strains (52). Within *Mlsa* strains, the gene (hitherto classified as untranslated) encoded a protein that profoundly affected the development of the αβ T cell repertoire, in particular by provoking deletion of Vβ8.1$^+$ cells (53). Conversely, Vβ8.1$^+$ cells from non-*Mlsa* strains were

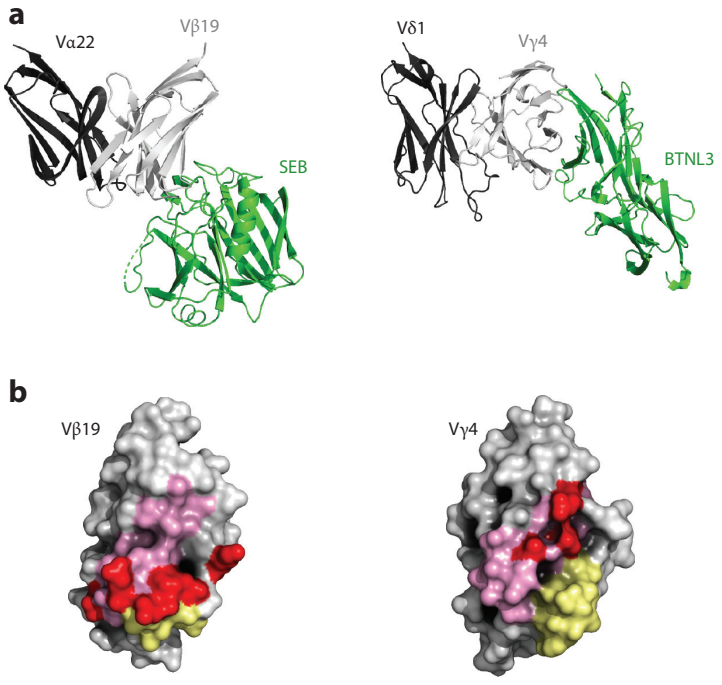


Figure 2

Comparison of Vβ-SAG and Vγ-BTNL interactions. (*a*) Cartoon representation (PyMOL) of a Vα22Vβ19 T cell receptor (TCR) binding SEB (*left*; PDB accession code 4C56) and a Vγ4Vδ1 TCR docked to BTNL3 (*right*; see **Figure 1**). Adapted from Reference 130. (*b*) Surface representation of the faces of the Vβ19 (*left*) and Vγ4 (*right*) domains that interact with their respective ligands. CDR2β/γ and HV4β/γ regions are highlighted in yellow and pink, respectively. Amino acids that were shown to directly interact with SEB or predicted to directly interact with BTNL3 based on molecular modeling and NMR data are highlighted in red.

acutely activated on exposure to the proviral antigen expressed by *Mlsa* strains. Moreover, as other such antigens were discovered, their expression in the thymic medulla was found to be responsible for the clonal deletion of several other Vβ subsets, depending on which antigens were expressed (54, 55). Additionally, exposure to proviral superantigens could induce anergy (56–58).

Prima facie, *Mlsa* resembles *Btnl1* and *Skint1* in having profound effects on the TCR repertoire, although it deletes rather than positively selects. Nonetheless, the physiologic significance of this remains unclear, possibly reflecting a remnant of immunoevasion by infectious virus. Consistent with this, other examples of endogenous superantigens so far identified are also proviral, including some encoded by MMTV, each targeting one or more specific Vβ subsets (59); these include the product of a human endogenous retrovirus, HERV-K18, which targets $V\beta7^+$ and $V\beta13^+$ cells (60).

In sum, there is as yet no known nonviral endogenous gene product analogous to the BTNL/Btnl proteins that might drive counterpart selection of αβ T cells expressing defined TCR V regions. Indeed, it seems intriguing that in cases where TCRα/β chain usage is limited—e.g., Vα14-$J\alpha18^+$ mouse iNKT cells; Vα24-$J\alpha18^+$ human iNKT cells; and human MAIT cells, which primarily express Vα7.2 recombined with Jα33, Jα12, or Jα20—the repertoire limitation is of Vα-gene segments, rather than of Vβ segments, for which there is stronger evidence for superantigen regulation. Indeed, although superantigens can skew the TCRα repertoire (61), structural and other

data argue that the restricted Jα-segment usage of NKT and MAIT cells reflects repertoire selection imposed via adaptive antigen-combining sites to which germ line–encoded CDR1 and CDR2 residues make critical contributions (62). The same may be true for the human T cell response to mycobacteria, which is enriched in usage of germ line–encoded TCRs (so-called GEM-T cells) that disproportionately use Vβ6–2 and Vβ30, but that additionally have highly restricted Vα usage (63–65). Likewise, the selection of the conventional polyclonal αβ T cell repertoire by polymorphic MHC shows allelic influence on the Vβ repertoire that may also be fully attributable to germ line CDR1 and/or CDR2 contacts with MHC within the antigen-combining site. In sum, there is as yet no clear evidence for TCRαβ repertoire selection resulting from V region–mediated innate interactions that are independent of the clonotypic antigen-combining site. Nonetheless, this does not preclude the contribution of such interactions to other components of αβ T cell repertoire regulation, as will now be considered.

LESSONS FROM MICROBIAL SUPERANTIGENS

To date the more numerous and better-understood superantigens are those encoded by viruses and microbes, most likely as a means of countering relevant immune responses. Given that endogenous proviral superantigens have particularly profound impacts on the T cell compartment, it is perhaps surprising that relatively few virus-encoded superantigens have been identified, examples being rabies virus nucleocapsid protein N (66), an as yet unidentified Ebola virus protein (67), and muLV gp30 (68). HIV-1 was reported to deplete $CD4^+$ and $CD8^+$ T cells from several specific $V\beta^+$ subsets (69, 70), although such effects were not universally observed (71, 72).

By contrast, there are many bacterial superantigens, particularly those encoded by multiple genera of *Staphylococcus* and *Streptococcus*, but also including proteins encoded by *Yersinia* and *Mycoplasma*. These superantigens are heterogeneous in the T cells that they target, in their structures, in their modes of action, and in the responses that they induce in T cells expressing relevant TCRβ or TCRα chains (73). Illustrating this, staphylococcal and streptococcal superantigens have been segregated into five classifications (**Table 1**).

For staphylococcal and streptococcal superantigens, it is generally the case that residues within a C-terminal β barrel together with additional N-terminal residues bind to the TCRβ chain via CDR2, but with additional, albeit variable involvements of germ line–encoded residues from HV4, from FR3 but outside of HV4, from CDR1, and from FR4 (which lies C-terminal to CDR3 and is encoded by Jβ) (**Figure 2**). Additionally, SpeC binds to CDR3 (73), arguing prima facie that its effects are not wholly innate, a point returned to in the next paragraph. Many superantigens show highly sequence-specific binding to Vβ, whereas others, such as SEB and SEC3, are broadly cross-reactive, engaging a tertiary conformation adopted by CDR2+HV4 and thereby inducing nonclonal responses from T cells expressing many different Vβ chains (74) (**Table 1**). In either case, the germ line–encoded sequences of the TCR Vβ regions can be considered as akin to microbial pattern recognition receptors (PRRs).

In addition to binding to TCRβ, staphylococcal and streptococcal superantigens contain an N-terminal β grasp that in most cases binds nonpolymorphic regions of the α chain of MHC-II with varying affinities (**Table 1**): Indeed, some superantigens, e.g., SEA, contain both low-affinity and high-affinity MHC-II interaction sites. There is mostly no requirement for MHC-II to be complexed with specific peptides (75), and superantigens such as SpeC bridge the TCR to MHC-II, independently of peptides for which the TCR is specific. Hence, although the CDR interface with MHC-II is affected, the aggregate outcome is that the clonotypic response mode is overridden by an innate interaction that elicits nonclonal responses from a broad swathe of the T cell compartment.

Table 1 Five groups of bacterial superantigens[a]

Superantigen group	Genera	Examples	TCR regions	Vβ specificity	MHC affinity	TCR/MHC contact	Peptide dependent
I	*Staphylococcus*	TSST-1 (singleton)	CDR2, FR3	High	Low	No	Yes
II	*Staphylococcus*	SEB, SEC	CDR2, HV4	Low	Low	Yes	No
	Streptococcus	SpeA	CDR2, HV4, CDR1	Medium			
III	*Staphylococcus*	SEA	CDR2, HV4, FR3 (CDR1, FR4)	High	Low + high (2 sites)	Potentially	No
IV	*Streptococcus*	SpeC	CDR1/2/3, HV4	High	High	No	No
V	*Staphylococcus*, *Streptococcus*	SEI, SEK, Spel	CDR2, FR3, FR4	High	High	No	No

Abbreviations: TCR, T cell receptor; MHC, major histocompatibility complex.

[a]Summary of the main characteristics differentiating the five main groups of staphylococcal and streptococcal superantigens. Vβ specificity summarizes the number of different Vβ TCRs engaged (high, very restricted repertoire; low, broad repertoire). Group III superantigens engage MHC-II molecules via two distinct sites, presumably leading to MHC-II cross-linking. TCR/MHC contact denotes whether there is superantigen-independent engagement of MHC by the TCR in addition to superantigen binding to the TCR and to MHC-II, respectively. Adapted from Reference 73, with additional information based on more recent reports cited in the text.

This may hold important lessons for BTN-mediated regulation of human phosphoantigen-reactive Vγ9Vδ2 T cells (**Figure 3*a*,*b***). First, it is possible that one domain of one of the BTN3A1/BTN3A2/BTN2A1 proteins engages Vγ9 HV4+/−CDR2 while another domain of one of the other proteins engages the CDR face of Vγ9Vδ2, somewhat equivalent to how MHC-II can contribute to superantigen activity. Such a model is consistent with the fact that a molecule encoded on human chromosome 6 (i.e., BTN2A1) is required in addition to BTN3A1+3A2 for mouse cells to support the phosphoantigen-mediated reactivity of human Vγ9Vδ2$^+$ cells (20, 76).

While such a composite interaction would be sufficient to trigger Vγ9Vδ2 T cells, the individual interactions of BTN3A1/2 with regions of the TCR might be of insufficient affinity to be detected biochemically by conventional approaches (77, 78). A similar situation might hold for Skint-mediated regulation of 17D1$^+$ DETC progenitors (see above). Moreover, this raises an important question as to whether or not BTNL3+8 and Btnl1+6 also bind third-party molecules, including other BTNL/Btnl proteins, and may thereby be bridged to the CDR3-containing faces of Vγ4$^+$ TCRs and Vγ7$^+$ TCRs, respectively. Note, however, that although this possibility formally exists, Vγ4$^+$ and Vγ7$^+$ T cells could respond to BTNL/Btnl molecules largely agnostic of the cell type expressing them (21).

SUPERANTIGEN BIOLOGY AND INNATE SIGNALING

The potency of superantigens such as staphylococcal TSST-1 to induce peripheral T cell activation and acute cytokine release (79) can be so strong as to cause toxic shock syndrome, which is associated with several settings of bacterial infection (80). Superantigens can also profoundly affect unconventional αβ T cells, including MAIT and NKT cells (81, 82), added to which their potency may be augmented by effects on other cell types, particularly those expressing MHC-II (83).

Just as the impact of high-affinity peptide-MHC (pMHC) complexes on TCRαβ varies according to the state of the T cell, e.g., thymocyte deletion versus strong activation of mature peripheral

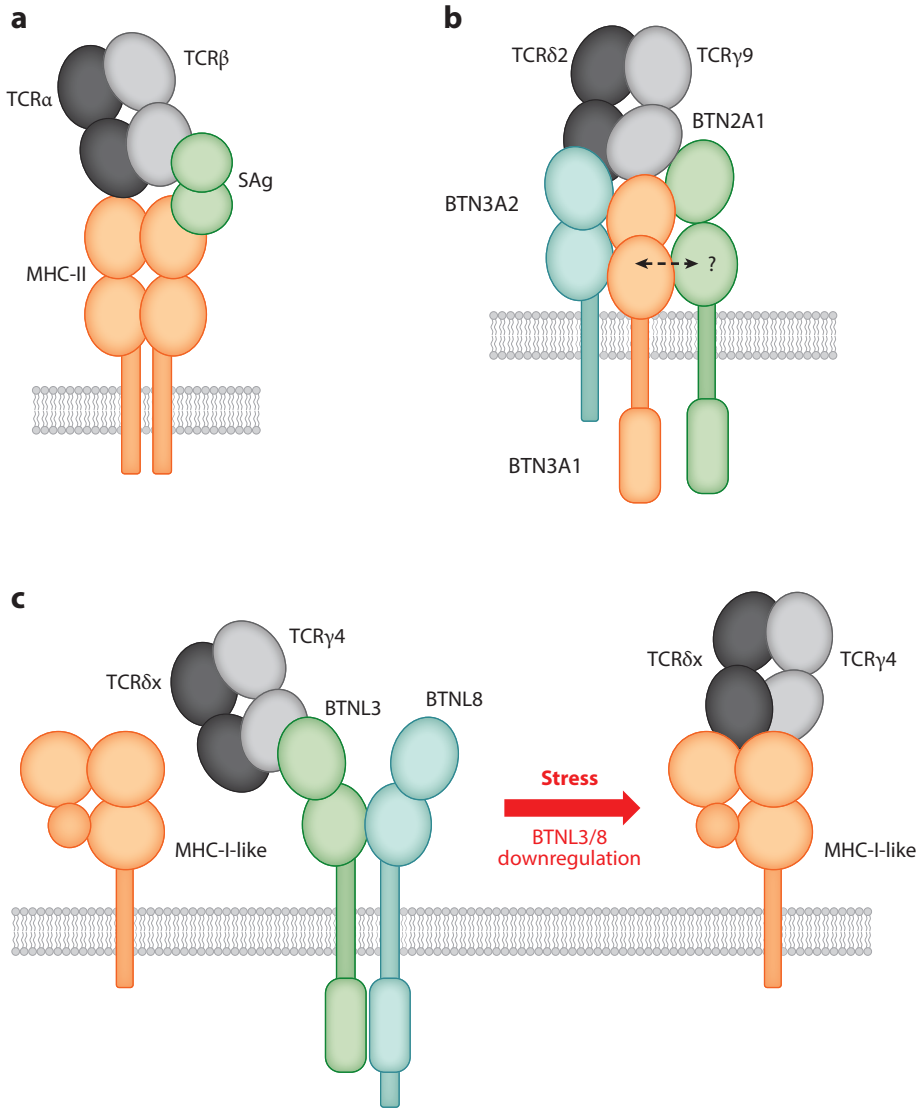


Figure 3

Different modes of innate reactivity of T cell receptors (TCRs). (*a*) Bridging of MHC-II and TCRαβ by a superantigen (SAg). Depending on the superantigen considered, the complex may or may not include a third interface between the TCR and MHC-II molecules. (*b*) Hypothetical simultaneous engagement of BTN3A1/BTN3A2/BTN2A1 by phosphoantigen-reactive Vγ9Vδ2 TCR. The dashed arrow and question mark indicate potential interactions between the BTN3 complex and BTN2A1. (*c*) Hypothetical model of a molecular switch dictating the mode of response of $V\gamma4^+$ TCRs. In normal human intestinal epithelium, $V\gamma4^+$ TCRs engage BTNL3+8, via CDR2γ–HV4γ, leading to a maturation/tonic signaling response. In conditions of stress, BTNL3+8 downregulation may allow $V\gamma4^+$ T cells to switch to an adaptive reactivity mode, e.g., recognizing MHC-I-like ligands, such as EPCR and CD1, presumably via classical engagement of CDR3 regions. This model may also apply to murine intestinal $V\gamma7^+$ T cells.

T cells, so the impact of superantigens on CD4$^+$ T cells varies according to their differentiation state, with strong activation being induced in naive T cells and anergy induced in memory T cells (84). Likewise, SEB, which binds CDR2+HV4, was recently shown to trigger massive cytokine release by MAIT cells, whereupon the cells rapidly adopted an exhausted phenotype (82). Clearly this too may inform Btnl biology, such as the differential effects of *Btnl1* gene expression during and after the establishment of the murine Vγ7$^+$ IEL compartment (see above). In this regard, it became evident early on that superantigens trigger unique signaling pathways, thereby offering the potential for different functional outcomes vis-à-vis adaptive responses of TCR$\alpha\beta$ (85, 86).

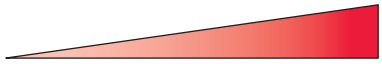
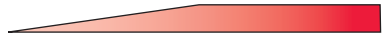
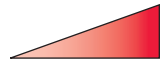
More specifically, T cell stimulation by bacterial superantigens has been proposed to depend on Gα11 and PLCβ rather than on the TCR-proximal kinase, Lck (87–89), which was reported to negatively regulate superantigen-mediated responses (90). It will be intriguing to consider how TCR-proximal signalosomes might be differently deployed according to the nature of TCR engagement, although there is strong precedent in B cell biology for differential outcomes being dictated by the form in which antigen is engaged. Additionally, signaling might be influenced by additional molecules that function de facto as costimulators, overriding a need for Lck-transduced signals. For example, SEE (and possibly also SME-Z) was proposed to promote Gα signaling by engaging LAMA2, which is the extracellular domain of the laminin A receptor, coupled to a G protein–coupled receptor (91). The capacity of superantigens to induce different signals from those induced by pMHC may hold lessons for BTNL/Btnl-mediated innate activation of Vγ4$^+$/Vγ7$^+$ T cells. Whereas full T cell activation in response to innate interactions with BTNL/Btnl proteins expressed at steady state would not be appropriate, full activation would be appropriate in response to adaptive ligands, such as EPCR, expressed by dysregulated cells (**Figure 3*c***).

The induction of anergy in memory CD4$^+$ T cells by superantigens was reported to involve Fyn, another TCR-proximal tyrosine kinase (84, 92). Unusually, however, this did not induce downstream Zap70 activation, thereby interrupting the conventional route by which TCR engagement activates T cells (92, 93). Interestingly, an analogous interruption may account, at least in part, for the inhibitory impact on TCR signaling imposed by PD-L1–PD-1 engagement (94). One consequence of that is the upregulation of NR4A transcription factors that have thereby been associated with $\alpha\beta$ T cell exhaustion in response to chronic signaling, e.g., within tumors (95). Intriguingly, *Btnl1*-selected mouse $\gamma\delta$ IELs and *Skint1*-selected mouse DETCs are also characterized by high *Nr4a* expression (18, 96, 97).

Hence, whereas nothing is currently known about how BTNL/Btnl proteins induce signal transduction downstream of the $\gamma\delta$ TCR, they may function analogously to PD-L1, a molecule with which they are structurally similar; but they may do so via innate interactions with the TCR rather than via innate interactions with a dedicated receptor, PD-1. Rather than being exhausted, *Btnl1/Skint1*-regulated $\gamma\delta$ T cells appear to have been placed into a state that is resting but that is fully competent to respond rapidly to appropriate stimuli. Such a reversibly anergic state may usefully describe the outcome of PD-1 engagement by PD-L1 under physiologic circumstances and moreover was recently considered as a preface to B cell activation (98).

In sum, TCR$\alpha\beta$ clearly has an intrinsic innate biology, evident from the interaction of germ line–encoded sequences with superantigens and from the capacity of those superantigens to induce distinct signal transduction pathways that elicit responses from discrete swathes of the T cell repertoire, as opposed to clonally restricted, antigen-specific responses (**Table 2**). By several biologic and biochemical criteria considered here, superantigen biology parallels the BTNL/Btnl-dependent biology of TCR$\gamma\delta$. This in turn suggests that innate interactions of TCRVβ and/or TCRVα chains might underpin one or more physiologic aspects of $\alpha\beta$ T cell regulation that have been pathogenically co-opted by microbial superantigens.

Table 2 Degrees of innateness of different antigen receptors[a]

	TCRγδ				Immunoglobulins			TCRαβ		
	Vγ4Vδ5 EPCR	Vγ9Vδ2 PAg-BTN3A1/2	Vγ9 SEA	huVγ4/moVγ7 BTNL3+8/Btnl1+6	V_H anti-HA stem (affinity matured)	V_H anti-HA stem (UCA)	Natural antibodies	pMHC	Vβ SpeC	Vβ SEB
Predominantly germ line determinants	–	?	+	+	–	+	+	–	+	+
CDR3 independent	–	–	?	+	–	–	–	–	–	+
CDR3 mostly uninvolved	–	–	–	+	–	–	–	–	–	+
Nonclonal response	–	+	+	+	–	+	+	–	+	+
Innateness										

Abbreviations: HA, hemagglutinin; PAg, phosphoantigen; pMHC, peptide-MHC; TCR, T cell receptor; UCA, unmutated common ancestor.

[a]Examples of systems in which TCRγδ, immunoglobulin, and TCRαβ show different contributions of innateness, as judged by the criteria in Column 1.

Certainly, there are several aspects of TCR-dependent αβ T cell regulation that are nonclonotypic. For example, whereas naive TCRαβ$^+$ CD8$^+$ T cell expansion requires recognition of cognate pMHC, cell survival in the steady state requires only the correct MHC-I molecule, while memory CD8$^+$ T cell survival can be sustained independently of peptide by nonspecific MHC-I molecules (99). Nonetheless, such nonclonotypic TCR-dependent responses might simply reflect cell differentiation state-dependent changes in the quantitative response to antigen engagement at the CDR1–3 face, rather than reflecting cell survival mediated by superantigen-like innate responsiveness to nonpolymorphic regions of MHC-I. Indeed, to date, no endogenous gene product (BTNL/Btnl counterparts) has been identified that might mediate physiologic, nonclonal, TCR-mediated aspects of αβ T cell biology.

This notwithstanding, it seems plausible that the PRR capacities of germ line–encoded subregions of TCRVβ and/or TCRVα chains, including CDR2+HV4, might permit the physiologic regulation of the TCRαβ repertoire by commensal-associated molecules. Such a process might, for example, exploit defined commensals to preemptively enrich for αβ T cells expressing TCRVβ and/or TCRVα chains highly suited to protection against pathogens that thrive in the same environment. This could be considered analogous to the selection of presumably useful gut γδ T cell compartments by butyrophilin-like proteins. Furthermore, the prospect that superantigen pathobiology reflects the exploitation of an existing physiologic pathway of innate TCRβ signaling is supported by at least one example wherein a bacterial superantigen appears to exploit the innate biology of TCRγδ.

SUPERANTIGEN BINDING TO TCRγδ

Staphylococcal superantigen, SEA, enters into high-specificity interactions with several germ line–encoded sites on Vβ5.2 (100). Additionally, however, SEA-pulsed B cell lines were killed by phosphoantigen-reactive human Vγ9Vδ2 T cells, and some clones of Vγ9Vδ2 T cells proliferated in response to SEA (101). By contrast to phosphoantigen reactivity, SEA responsiveness required that the Vγ9Vδ2 T cells interact with MHC-II$^+$ cells, although there was no requirement for

MHC-I in either case. Furthermore, unlike the dependence of phosphoantigen reactivity on both Vγ9 and Vδ2, SEA reactivity required only Vγ9 (note that Vγ9 is termed Vγ2 in the primary source paper). The responses mapped to the binding of SEA to germ line–encoded motifs of Vγ9, most likely CDR2 and HV4. The interactions showed high specificity, eliciting >1,000-fold-stronger responses than did SEE, which is >80% identical to SEA, while none of 11 other superantigens tested elicited any responses. Interestingly, binding was essentially independent of SEA C-terminal sequences that are required for TCRβ binding, instead utilizing N-terminal residues that are contiguous in tertiary conformation with the C-terminal Vβ binding site, and whose counterparts in SEB and SEC3 form contacts with murine Vβ8 via CDR2, and variably via CDR1, FR3, and HV4 residues independently of CDR3.

Given that discrete HV4γ mutations abrogated the BTN3A1/3A2/2A1-dependent Vγ9Vδ2 response to phosphoantigen (see above), it can be considered that SEA exploits a binding site that may mediate innate interactions between the TCR and an endogenous regulator, namely a BTN protein. Moreover, the fact that SEA binds TCRVβ and TCRVγ sequences could be considered consistent with some evolutionary similarities of Vβ and Vγ chains: Thus, related to the organization of the TCRδ locus within the TCRα locus, the translation products of 10 mouse V-gene segments and 5 human V-gene segments can be used interchangeably as Vα or Vδ, and hence can pair with either Vβ or Vγ (102). Based on the reactivities of SEA, the evolutionary similarities may extend to the use of germ line–encoded Vγ/Vβ regions for functionally important innate interactions. As pointed out by Morita and colleagues (101), the same rationale may extend to human IgV_H4 (and possibly IgV_H3), which reportedly mediates responses to superantigen SED (and possibly SEA), including via IgV_H-FR3/HV4 residues (103, 104). Such interactions may offer a window onto a physiologically important innate biology of B cell receptors (BCRs) (**Table 2**).

NATURAL ANTIBODIES

Identifying strictly innate contributions of IgV regions to immune responses is challenging because the relevant sequences are frequently altered by somatic mutation (98). This notwithstanding, there are examples of germ line motifs, particularly IgV_H CDR2, critically contributing to antibody binding. For example, following infection of mice with a spectrum of pathogens, e.g., *Streptococcus pneumoniae*, the host is critically and rapidly protected by so-called natural antibodies that are polyreactive to microbial determinants, such as capsular polysaccharides, as well as to self-molecules such as phosphatidyl choline (PC) (105, 106). The primary producers of such antibodies are B-1 B cells (106), which in mice have been subdivided into two subsets (B-1a and B-1b) that are considered, respectively, to be sources of preimmune natural antibodies and natural antibodies rapidly induced by infection and/or immunization: Both are important (107). Interestingly, by aiding the clearance of apoptotic debris, PC-reactive antibodies can also play an important role in maintaining steady-state homeostasis, another function with which the innate responses of γδ T cells have been associated (31).

Because natural antibodies are largely germ line, with little junctional diversity and infrequent somatic mutation, and because they provide rapid protection, they are understandably compared to PRRs (108). Like PRRs, the relevant germ line sequences may be under stringent heritable selection for their capacity to contribute to the innate biology of BCRs. In support of this, computational studies underpin the prospect that germ line antibody sequences comprise multiple sequence motifs that on aggregate form effective polyspecific binders (109). Moreover, antigen-receptor signaling in B-1 cells is distinct from that in conventional B-2 B cells, as reflected, for example, in its dependence on Ras guanyl nucleotide–releasing protein 1 (RasGRP1) (110), and B-1 B cells display constitutive, low-level antigen-receptor-dependent signaling, akin to that reported

for *Skint1*-selected DETCs (36). This notwithstanding, structural studies of antigens complexed to natural antibodies have highlighted critical contributions made by CDR3 regions, strictly distinguishing this biology from the CDR3-independent innate engagement of BTNL/Btnl proteins or superantigens (111).

INNATE INTERACTIONS OF IGV$_H$

Other scenarios in which germ line IgV$_H$ CDR2 residues contribute critically to B cell responses have emerged from studies of protective human B cell responses against influenza and malaria. Thus, human antibodies utilizing V$_H$1–69 were prevalent in screens for reactivity to the influenza hemagglutinin (HA) stem, which, being less variable than HA head groups, is a vaccine candidate for cross-group protection against multiple virus strains. V$_H$1–69 was prevalent whether the screen was of volunteers recently vaccinated with seasonal influenza, or of phage display recombinant antibodies from nonimmune subjects (5, 112). Structural data confirmed a dominant role of IgV$_H$1–69 vis-à-vis IgV$_L$ in binding to the HA stem. Moreover, when lineage trees were constructed of 197 anti-H1 stem antibodies developing over time in a single individual (113), it was possible to trace initial reactivity of many antibodies to a phenylalanine (F) residue at residue 52, within CDR2. Indeed, the unmutated common ancestor (UCA) of several highly mutated antibodies bound to the stem entirely via H-chain contacts, among which 52F was directly involved.

At the same time, the UCA featured a favored V$_H$1–69 gene rearrangement that was denoted by a conserved tyrosine at residue 98 within CDR3, indicating again that purely innate antigen reactivity was critically integrated with adaptive clonal restriction. This notwithstanding, residue 52F is polymorphic, present in 8/14 known V$_H$1–69 alleles, and individuals homozygous for leucine at residue 52 (found in 6/14 alleles) displayed lower titers of antistem antibodies, which mostly used other V$_H$ segments. Thus, one may consider that exposure of IgV$_H$1–69.52F$^+$ individuals to influenza promoted the activation of high numbers of V$_H$1–69$^+$ B cells, creating a large precursor pool for subsequent affinity maturation and clonal expansion. In that regard, it seems noteworthy that 52F became superfluous in highly mutated antibodies displaying highly evolved adaptive CDRs: It had done its job!

In parallel studies of HA-stem-reactive antibodies that broadly neutralized group 1 and group 2 influenza viruses, multiple donors showed key contributions of unmutated CDR2 residues from V$_H$6–1, particularly when rearranged to IgD$_H$3–3. There was frequent pairing with light chain, V$_K$3–20, albeit that the V$_H$ region again made most of the contacts (114). Moreover, in other classes of group 1– and group 2–cross-reactive antistem antibodies, a critical contribution was made by germ line CDR2 residue 53Y encoded by V$_H$1–18 (114). The authors considered that given the potentially immense diversity inherent in the Ig repertoire, canonical response patterns of the kind observed were unexpected and suggested a major role of germ line sequences in directing downstream maturation of the B cell antigen receptor response.

Of note, similar principles pertain to malaria. Following immunization with radiation-attenuated *Plasmodium falciparum* sporozoites and subsequent parasite challenge (115), several Tanzanian subjects displayed protective antibodies with dual specificity for two regions of the circumsporozoite protein (CSP): the NANP repeat and a peptide, NPDP, that joins the CSP N-terminal to the NANP repeat (116). Of note, the great majority featured V$_H$3–30-family gene segments encoding at position 52 within CDR2 a tryptophan (W) that makes direct contact with the NANP repeat (117), again creating the potential to prepare a large pool of V$_H$3–30-family Igs as an expanded template for subsequent affinity maturation. Indeed, V$_H$3–30-family Igs occurred at high frequency among IgM$^+$ B cells of vaccinees, as well as in malaria-naive individuals receiving the RTS,S subunit vaccine. Likewise, there was frequent usage of V$_K$1–5. Conversely,

V_H3–30 alleles encoding serine at 52 could not bind CSP, raising again the prospect that defined germ line sequences of discrete IgV_H alleles can confer profound selective advantages.

Viewed collectively, germ line residues of natural antibodies and those of UCAs of high-affinity antibodies may share a capacity to accelerate the development of host-protective, clonally restricted reactivities, by creating a state of preparedness akin to that considered above for the innate responses of TCRγδ. In the cases of antibodies, the biology integrates the contribution of strictly innate antigen recognition with major contributions from CDR3 residues, albeit frequently from germ line sequences. At the same time, the question is raised as to whether key germ line IgV_H-CDR2 sequences are maintained by engaging hitherto uncharacterized endogenous selecting ligands, or commensal-encoded determinants, as was considered above for TCRVβ-specific superantigens.

The role of endogenous ligands (i.e., autoantigens) in regulating B cell development has long been debated. Prior to IgV_L-gene rearrangement, B cells must traverse pre-B selection, at which point they must receive signals from a pre-BCR composed of IgV_H chains paired to a surrogate light chain, akin to pre-TCR-mediated selection of αβ T cell progenitors. There is evidence in both cases that signal transduction can be ligand independent (118, 119), but those data derive from experimental systems lacking scope for stringent intraclone competition. By contrast, it was claimed that under normal circumstances many pre-BCRs are de facto autoreactive, and that the signaling resulting from ligand engagement promoted selective B cell progenitor survival and/or expansion (120).

Thereafter, B-1 cell development is broadly accepted to depend on strong positive selection by autoantigens (121), and although the situation is more contentious for B-2 cells, there are striking examples of some level of autoantigen reactivity increasing B cell fitness within a polyclonal repertoire (108). Indeed, IgV regions contributed a very substantial competitive advantage in the peripheral B cell pool, relative to signaling-competent Ig constructs lacking V-domains, suggesting that tonic signaling was not necessarily ligand independent (122). Moreover, recent evidence argues that the trajectory toward high-affinity antibodies can be highly efficient if the UCA is autoreactive, but is then rapidly purged of autoreactivity (redeemed) by somatic mutation (98). In this context, too, autoreactivity would seem to establish a reversible anergy or state of preparedness, akin to the proposal of Jerne (123, 124), and as considered, above, for the impact of BTNL/Btnl proteins on human/murine gut γδ T cells.

Indeed, whereas the many considerations of pre-BCR or Ig autoreactivity focus on ligand engagement with the conventional CDR face, including CDR3, it may be the case that there are CDR3-independent engagements of endogenous regulators via a binding face composed of CDR2+/−HV4, akin to the interactions of BTNL/Btnl proteins with TCRγδ. In this context, Rosado & Freitas (122, p. 2690) wrote, "recognition interactions related to B cell survival may not require the involvement of the full antigen binding site and might be exclusively V_H mediated." Moreover, it is noteworthy that for B-1 B cells BTK-dependent signaling is required for the cells' autoantigen-dependent positive selection and activation, but not for the cells' survival (125).

As is often the case, useful insights may emerge from pathology. Thus, the immunoglobulins expressed by chronic lymphocytic lymphomas and by some other B cell malignancies are commonly limited to very few IgV_H regions (126). Thus, over one-third of human diffuse large B cell lymphomas (DLBCLs) of the ABC type include either V_H4–34 or V_H3–7, which mediate autoantigen engagements that are critical for the survival of the DLBCL cells (127). Such prevalence of discrete V_H segments argues for germ line sequence–mediated selection, and although the mature antibodies carried somatic mutations, it was evident at least in one case that the autoreactivity of V_H4–34 mapped extensively to CDR1, CDR2, and FR1, and that reversion to the UCA only moderately diminished reactivity (127). While this is not to dismiss contributions of CDR3

residues to the mature antibody, it fuels the prospect that IgV_H regions, like TCRγ and TCRβ, may interact with endogenous molecules by use of binding motifs outside of the CDR3-focused face.

The profound biological significance of innate IgV interactions distinct from those directly targeting antigen was supported by a recent report that homotypic interactions of antibodies bound to the repetitive NANP antigen of malarial CSP (see above) antigen were so important to the efficacy of the B cell response that the site was subject to affinity maturation (128). Thus, the germ line repertoires of antigen receptor V-regions may be selected based on a number of different factors that may include innate interactions, as seems likely for the TCRs of intestinal γδ IELs. Indeed, sea lampreys, which do not diversify Ig-SF genes, express at least one Ig family member that resembles VpreB (129) and that has presumably been selected on the basis of innate interactions.

This is not to suggest that there will exist discrete families of lymphocyte antigen receptor–selecting elements with the level of influence that MHC exerts on the αβ T cell repertoire. Rather, nonclonal, antigen receptor–dependent B and T cell responses might be driven by functionally redundant entities and/or commensal-associated, as considered above. At the same time, innate antigen receptor responses in different lineages might collaborate. In the case of malaria, for example, it seems tempting to link three observations: first, that mice show quasiadaptive, host-protective expansions of $V\delta6.3^+$ γδ T cells in response to *Plasmodium chabaudi* infection (42); second, that host-protective Ig responses of humans to malarial sporozoite vaccination seem frequently to be initiated by IgV_H-specific, germ line–encoded CDR2 interactions with antigen (117); and third, that the efficacy of malarial sporozoite vaccination was associated with γδ T cells (115). The added value of such collaboration may be an enhanced state of preparedness for mounting critical host-protective antigen-specific responses.

FUTURE PROSPECTS

The manner by which superantigens profoundly regulate αβ T cells alerted us some years ago to the prospect that innate sequence motifs in antigen receptor V-regions can provoke nonclonal responses of adaptive lymphocytes. Nonetheless, because normal physiologic counterparts of those interactions have not been elucidated, the innate biologies of antigen receptors have received relatively little attention. Added to this, the impact of superantigen and other germ line–directed responses reviewed here has seldom been considered as operating independently of the primary antigen-binding CDR that mediates the core clonotypic biology of adaptive immunity. However, recent data concerning BTNL/Btnl-dependent γδ T cell selection, taken in the context of many other unresolved questions considered in this review, suggest that as we go forward, our minds should be open to the possibility that various antigen receptor V-region-mediated, innate interactions with endogenous and/or microbial moieties may regulate T and B lymphocytes independently of, or in concert with, clonotypic responsiveness. In particular, such interactions may ensure that appropriate lymphocyte repertoires are placed into a state of preparedness for mounting efficacious immune responses. Thus, the identification of any molecular moieties that de jure engage germ line–encoded V-region sequences may offer routes to understanding the evolution of our antigen receptor repertoires and may provide novel means to manipulate subsets of lymphocytes, potentially for clinical benefit.

DISCLOSURE STATEMENT

A.C.H. is cofounder, equity holder, and board member of Gamma Delta Therapeutics and ImmunoQure AG; cofounder, advisor, and equity holder of Adaptate Biotherapeutics; member of the scientific executive board of CRUK; and consultant for eGenesis Bio.

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