(which produce neutralizing antibodies), there are numerous additional vaccine-induced responses of the innate and adaptive immune systems that may protect against infection and further viral immune escape. Conversely, there are uncharacterized mutations outside of S that could facilitate SARS-CoV-2 immune evasion.

The growing evidence for the emergence of immune escape mutations in protracted SARS-CoV-2 infection and for multiple, rapidly spreading variants should raise broad concern and action. Reducing the spread of SARS-CoV-2 is most likely to prevent further selection of immune escape variants. This will require a coordinated and comprehensive global vaccination and prevention strategy. Partial roll-out and incomplete immunization of individuals leading to suboptimal titers of neutralizing antibody could promote selection of escape variants that negatively affect vaccine efficacy. Increased genotypic and phenotypic testing capacities are essential worldwide to detect and characterize circulating SARS-CoV-2 variants that may emerge from selection by natural or vaccine-mediated immune responses. Infections that occur among vaccinated individuals should be aggressively evaluated for the mechanisms of breakthrough. The explosive, global spread of SARS-CoV-2 and the devastation it has wreaked is a stark warning of the potential for new variants to further complicate pandemic control. Vaccine manufacturers are now testing potential booster vaccines against circulating SARS-CoV-2 variants, and more broadly active monoclonal antibodies are in development for therapy. Such proactive approaches are likely to be needed to ensure pandemic control and elimination. ■

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IMMUNOLOGY

Unusual T cell receptor in opossum

The structure of a marsupial T cell receptor illustrates the emerging trend of noncanonical antigen binding

By **Michael F. Criscitiello**

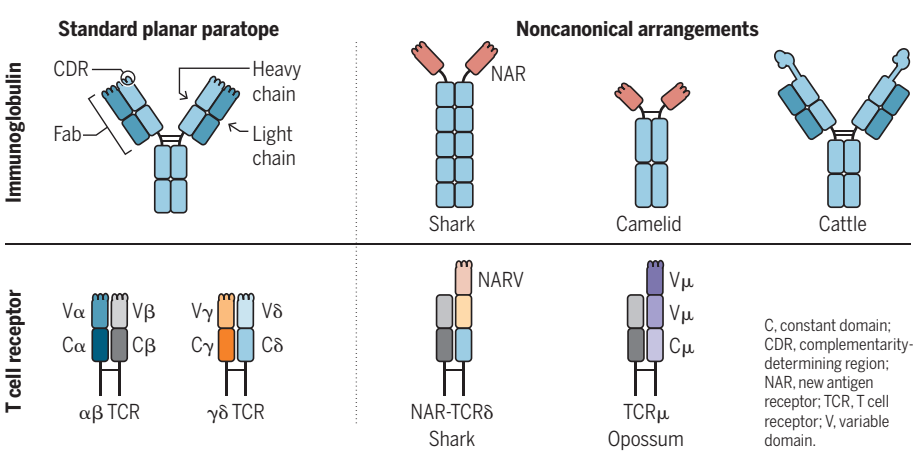
B and T lymphocytes of the vertebrate adaptive immune system have structurally, genetically, and evolutionarily related receptors for antigen recognition that initiate immune responses with notable specificity and memory. In general, the antigen binding sites of these receptors are evolutionarily conserved, yet a few very different immunoglobulin (Ig) structures have been characterized from shark, camelids, and cow B cells. On page 1383 of this issue, Morrissey *et al.* (*1*) reveal the structure of an opossum T cell receptor (TCR) that also eschews the vertebrate norm. This marsupial TCR is the latest in an emerging trend of smaller, projecting structural domains that are used for antigen recognition by the adaptive immune systems of some species, and it might have therapeutic potential.

From sharks to man, vertebrates have two varieties of T lymphocytes for orchestration of immune responses through cytokine secretion and direct killing of infected or cancerous cells. T cells of αβ and γδ lineages differentiate in the thymus where they rearrange genetic loci encoding either an αβ or a γδ heterodimeric TCR for antigen recognition. More than a decade ago, a fifth TCR chain in the older nonplacental mammals (including platypus, echidna, and marsupials), called TCRμ, was discovered (*2*). Although most similar to TCRδ, TCRμ is encoded at a distinct locus and was predicted to have two variable domains at its membrane-distal amino terminus. Little is known of the T cells that express TCRμ.

Morrissey *et al.* greatly extend our understanding of TCRμ and the cells that express it. Although the TCRμ chain was found not to be expressed in the peripheral blood mononuclear cells in opossums, nearly as many γμ T cells were found in the spleen as αβ-expressing T cells. In addition to confirming TCRγ as the heterodimeric partner of the TCRμ chain, single-cell RNA sequencing analysis showed that most of the γμ T cells use the CD8αα$^+$ homodimer, although some expressed neither the CD4 nor the CD8 TCR coreceptor. The more common CD8αβ$^+$ heterodimer is used by cytotoxic T lymphocytes. CD8αα$^+$ function is largely unknown, although in humans it can be an inhibitory coreceptor on natural killer cells (*3*). Functional studies will have to determine if TCRγμ signaling is inhibitory or more regulatory in nature.

Reaching for antigen

TCRγμ is part of a growing trend of T and B cell antigen receptors with reach. Most vertebrate antigen receptors bind antigen with six CDRs, three contributed by V domains of each partner chain (heavy and light chains, TCRα and β, or TCRγ and δ). TCRμ is the latest to break from this canon.



GRAPHIC: C. BICKEL/*SCIENCE*

The usual structure of the TCR is similar to one antigen binding arm (Fab) of Ig (including secreted antibodies) produced by B cells. The TCR and Fab of Ig are a heterodimer of two chains, each comprising a constant domain and a variable domain, which is diversified by somatic gene rearrangement. Three hypervariable loops from each variable domain interact with antigen differently in each lymphocyte, providing a repertoire of antigen recognition for the host's immune defense. The three hypervariable loops of each variable domain are called complementarity-determining regions (CDRs). CDR1 and CDR2 are encoded within a variable (V) gene segment; CDR3 is the product of the DNA junctions produced by the rearrangement of V, diversity (D), and joining (J) gene segments in lymphocyte development. Notably, the TCRγ variable gene rearrangements that paired with marsupial TCRμ showed restricted diversity compared with those pairing with TCRδ, often relying on microhomology in the ends of Vγ2 and Jγ3. This supports the idea that TCRγ variable domains may play a supportive role for the TCRμ chain rather than contribute to antigen recognition. CDR3 of the protruding membrane-distal TCRμ domain was longer and more diversified, which is suggestive of a role in antigen recognition.

The TCRγμ crystal structures reported by Morrissey *et al.* show a projecting μ variable domain with a relatively long and diverse CDR3, which is more similar to Ig variable domains in amino acid sequence and structure than to the variable domains of TCRs. Because the TCRγ chain does not provide a variable domain partner for the TCRμ membrane-distal variable domain, the authors rightly compare TCRμ to the Igs that do not use light chains: camelid single-domain antibodies (VHH) (*4*) and immunoglobulin new antigen receptor (IgNAR) from cartilaginous fish (*5*). These Igs have a lone variable domain jutting out without three adjacent CDRs provided by an Ig light chain (see the figure). The structures (*6*) of these single–variable domain paratopes (antigen binding sites) are being developed as immunotherapeutics, including for COVID-19 (*7*). These single-domain binders nearly have the specificity of traditional monoclonal antibodies but are less bulky so they can access sites and bind recessed epitopes. This may also be the case for marsupial TCRμ.

Another lymphocyte antigen receptor that appears to break the two–variable domain and six-CDR paradigm occurs in sharks. NAR-TCR comprises two variable domains and a TCRδ constant domain (*8*), possibly to yield a structure convergent upon that of marsupial TCRμ (*9*). Additionally, a textbook-defying antigen receptor was discovered in cattle, which contains a subset of ultralong Ig heavy chain CDR3 "cattlebodies" (*10*). Hyperactivation of AID (activation-induced cytidine deaminase) for somatic hypermutation diversifies CDR3 (*11*), which has produced broadly neutralizing cattlebodies to HIV when cows were immunized with gp120 (*12*).

The study of Morrissey *et al.* is a reminder of how conserved B and T cell antigen receptor immunogenetics are and how the interrelationships of antibodies and TCR are not only obvious in ancestral shark immunity but also in mammals. The antigen binding variable domain of the TCRμ paratope is similar to various single-domain Ig structures that have evolved in other species; whether they bind antigen similarly remains unknown. This perpetuates an emerging theme of Ig heavy chain variable domains being used on TCRδ in most vertebrate taxa, along with somatic hypermutation of TCR loci, demonstrating general plasticity between Ig and TCR immunogenetic components and diversification mechanisms (*13*). It is doubtful that these instances are as phylogenetically rare as they seem now. "Reaching," smaller, protruding paratopes will continue to be engineered into human antibodies, and perhaps one day the TCR as well.

Studies of thymic development of γμ T cells, identification of the antigens or ligands bound, and assays of cellular effector function will be needed to understand if this third TCR heterodimer also defines a third lineage of T cells. However, it is now clear that many taxa of diverse vertebrates have innovated the lymphocyte antigen receptor away from the relatively planar, six-CDR paratope to somatically diversify a smaller, more probing antigen binding surface. Human malaria patients can also make similar single-domain antibody paratopes (*14*). Perhaps these TCRs with reach will also be suited for immunotherapeutics. ■

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IMMUNOLOGY

Catching the wave

Uncoupling metabolism from cytoskeletal regulation leads to T cell dysfunction

By **Sophie Hambleton**

A central paradox in many inborn errors of immunity is the conjunction of autoimmune and autoinflammatory pathology with susceptibility to infection. On page 1333 of this issue, Liu *et al.* (*1*) explore the molecular mechanisms of immune dysfunction in mice whose T cells lack expression of the cytoskeletal regulatory protein WAVE2 (Wiskott-Aldrich syndrome protein family member 2). Without WAVE2, T cells do not move around normally, interact closely with antigen-presenting cells, nor generate protective immune responses (*1*–*3*). Nonetheless, they are adept at infiltrating nonlymphoid tissues and exhibit dysregulated proliferation and pro-inflammatory effector function, associated with excessive activity of mechanistic target of rapamycin (mTOR). Treatment of WAVE2-deficient mice with an mTOR inhibitor restored T cell quiescence and immune homeostasis. The description of WAVE2 as a negative regulator of mTOR, the master regulator of lymphocyte metabolism, draws attention to an underexplored, and eminently druggable, candidate mechanism for inflammatory complications of immunocytoskeletal disorders.

To do their job effectively, T cells need to move to the correct location, sense antigen by forming an immunological synapse (IS), and respond appropriately. This requires dynamic reorganization of the actin cytoskeleton at the IS to support the receipt of an array of cell-associated and soluble signals, as well as the polarized delivery of effector responses such as cytotoxicity molecules and cytokines (*4*) (see the figure).

Downstream of T cell receptor (TCR) signaling, WAVE2 is activated by the cell membrane–associated small guanosine triphosphatase RAC1 and nucleates actin branching by actin-related protein 2 (ARP2)–ARP3 (*2*). Assembly of the branched actin network

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