From basic research to clinical application of γδ T cells

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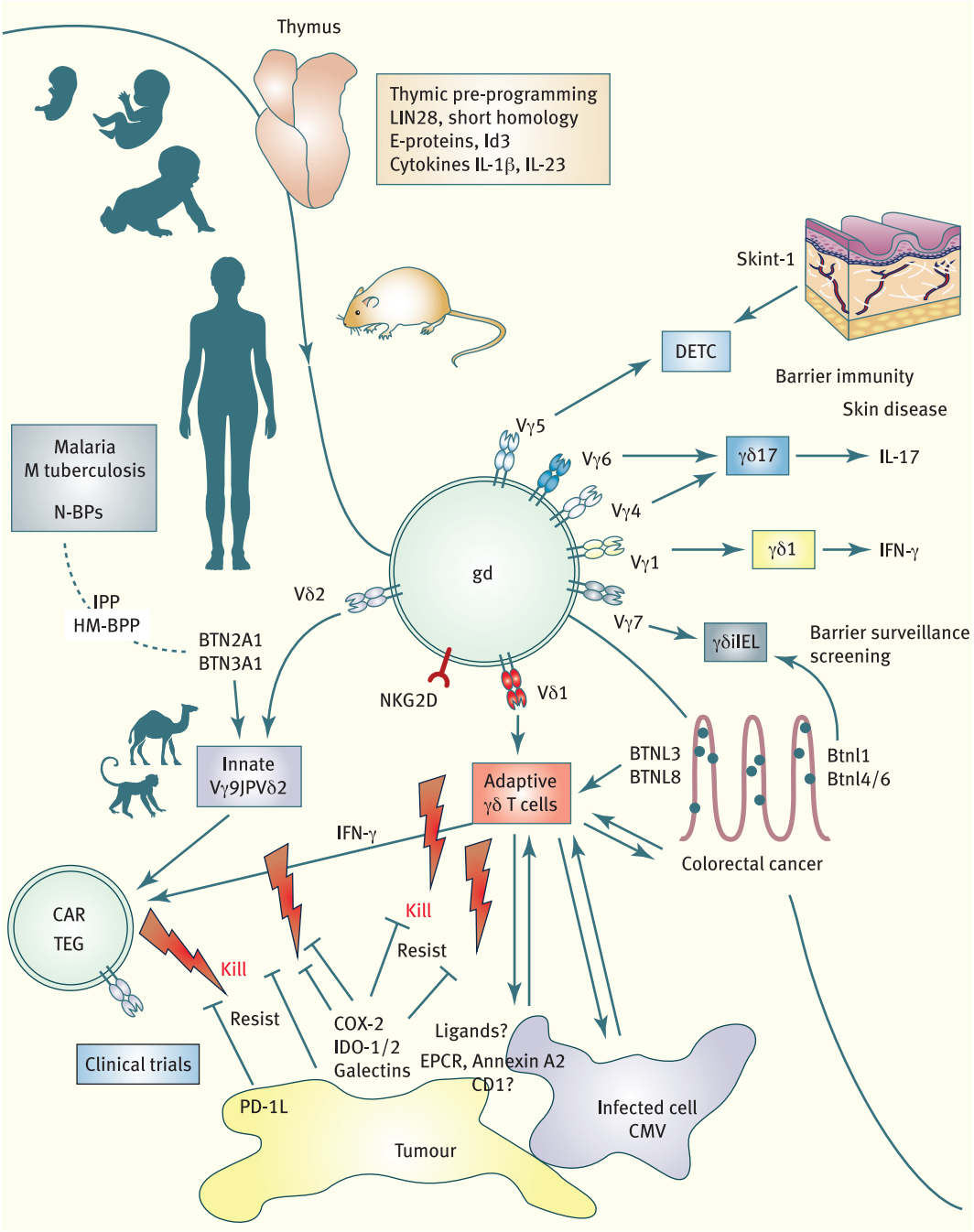
1 INTRODUCTION

Uncovering the mysteries of γδ T cells generates a flourishing enthusiasm that provided major progress in our understanding of their biology during the recent years. It was thus timely to release a new special edition on this topic in *Immunological Reviews*. This edition on γδ T cells was the initiative of our departed colleague Pr Wendy Havran who deceased suddenly in January 2020. She was one of the pioneers of γδ T cell field and a major contributor to the knowledge we are now all sharing on these cells. A heartfelt tribute to Wendy by Jameson and colleagues is thus opening this edition.

γδ T cells represent the third cell lineage of lymphocytes expressing a highly variable antigen receptor generated, as for αβ T cells and B cells, through V(D)J gene rearrangement. Their T cell receptor (TCR) repertoire can be highly diverse but also includes subsets with invariant or semi-invariant TCRs. Although γδTCR structure and signaling are similar to αβTCR, the different nature (not peptide-MHC complex) and diversity of the antigens recognized by the γδ TCR makes it, in that respect, more akina B cell receptor (BCR). γδ T cells are thus considered an atypical lymphocyte subset, enriched in non-lymphoid tissues, at barrier surfaces where they are recognized as playing a frontline immune surveillance function. Amazingly, in 2007, when Wendy Havran and Mitchell Kronenberg introduced a previous *Immunological Reviews* special edition on γδ T cells they declared: "*Immunologists are splitters rather than syn-thesizers. With the power of multicolor flow cytometry and other methods, immunologists have divided the lymphocytes into ever more separate populations"*.[1] Thirteen years later, this sentence makes even better sense. With the emergence of multicolor flow cytometry, CYTOF, scRNASeq, NGS TCR sequencing, etc, we now have access to the most precise details of γδ T cell heterogeneity and complexity. However, these ad-vances allow us to start understanding the role of γδ T cells in immune responses to infection and cancer. As presented in many of the reviews composing this edition, novel technologies have certainly contributed to our current understanding of γδ T cell development, tissue localization, migration, and functions in immunity and beyond. Last decade also brought critical information on γδ TCR ligands (recognized as antigens or as selection/ homing molecules) and on the innate-like or adaptive-like responses of γδ T cells. This volume comprises papers that encompass the recent findings obtained on γδ T cells from their phylogeny to the development of new γδ T cell-based immunotherapies.

2 γδ T CELL PHYLOGENY

αβ T cells, γδ T cells, and B cells can be found in all classes of jawed vertebrates and phylogenetic studies have highlighted the importance of the three "adaptive" lymphocyte lineages across evolution in verte-brates. Pioneering studies in jawless vertebrates by Max Cooper and Thomas Boehm provided extensive characterization of two T cell-like and one B cell-like cells, clonally expressing three types of variable lym-phocyte receptors (VLRs), structurally distinct from the TCR and the BCR.[2] Interestingly, one type of VLR (VLRC) is expressed by a T-cell line-age with localization in epithelia, transcriptomic profile, and functional properties analogous to γδ T cells. These mechanisms of highly variable antigen receptor generation expressed by three distinct lineages pre-served over a 450 million of years represent an additional indication that γδ T cells are probably an important component of an efficient immune system.



These phylogenetic considerations are reviewed by Hermann and colleagues, as well as the identification of pAg-responding Vγ9Vδ2 T cells in other species than primates, such as alpaca. Their review supplies an original perspective on the similarities and differences between γδ T cells from various species, and on how these stud-ies can help deciphering the molecular determinants of pAg recognition by the Vγ9Vδ2 TCR.

3 DEVELOPMENT OF γδ T CELLS IN THE THYMUS AND FUNCTIONAL PREPROGRAMING

γδ T cells are the first T lymphocytes generated during both human and mouse ontogeny, not at least because they do not need to undergo extensive positive and negative selection related to MHC presented peptides. Like αβ T cells, γδ T cells develop in the thymus from precursors arising from fetal liver of from the bone marrow. Understanding the development of γδ T cells in the thymus has been the subject of many studies since the discovery of γδ T cells in the eighties. Although many issues remain controversial or unclear, all these studies indicated that γδ T cell development sharply differs from that of αβ T cells.

During fetal and neonatal life, distinct waves of development give rise to distinct subsets of γδ T cells categorized by Vγ and Vδ TCR chain usage, by functional programming to ef-fector cells already in the thymus and by the different peripheral tissues that they populate after leaving thymus. Within the thymus, different molecular signals are involved in the orientation of T cell progenitors into the αβ or γδ T cell lineage, the most consensual being the differential signal strength of pre-TCR or γδ TCR signaling, respectively. Many other signals are involved in this process as well as in the commitment toward specific functional properties, particularly the capacity to produce IL-17 versus IFN-γ. On this topic, Anderson and colleague discuss a two stage model involving the E Protein-Id axis regulated by γδ TCR signaling.[5] This models implies specific thymic signaling niches segregated in time and space that are required for IL-17-producing versus IFN-γ-producing γδ T cell de-velopment. In agreement with the idea that γδ thymocytes receive distinct combinations of γδ TCR and environmental cues such as cytokines and co-stimulatory signals for functional differentiation, Silva-Santos and colleagues present the key cellular events of this developmental preprogramming in the mouse thymus.[6] They also describe the extrathymic molecular signals that regulate effector function maintenance versus plasticity in the periphery, with a special focus on gene regulation by chromatin remodeling and miRNA. Most of these studies have been done in mice, but Vermijlen and colleagues have recently delivered important results on the development of human γδ T cells, particularly using high throughput TCR sequencing.[7] In their review, they present and discuss the similarities between mouse and human γδ T cells. This team explains that an innate and (semi)-invariant γδ TCR repertoire is generated in early life, both in humans and mice, together with thymic functional programming (described above). This repertoire plays an important role against infections during the pre- and peri-natal period, such as during CMV or toxoplasmosis fetal infections.

4 FUNCTIONS OF IL-17-PRODUCING γδ T CELL S AND MIGRATORY PROPERTIES AT BARRIER SURFACES

Production of IL-17 is probably one of the most studied function of mouse γδ T cells during the recent years, especially in disease models. At the same time, our understanding of human IL-17-producing γδ T cells is still fragmentary. The cytokines IL-17A and IL-17F are critical in the fight against infectious agents but has also detrimental effects in cancer and inflammatory diseases. In mice, γδ T cells often largely outnumber αβ T cells as a source of IL-17 in diverse pathological settings. Understanding the functions and regulation of IL-17-producing γδ T cells, aka γδT 17 or γδ17 cells, is thus key to get insight into the pathophysiology and treatment of many diseases. O'Brien and Born provide an extensive description of and reflection about IL-17 producing murine γδ T cells, from their development to peripheral functions.[8] They compare the two major IL-17-producing γδ T cell subsets in mice: the Vγ6Vδ1$^+$ and Vγ4$^+$ T cells. Although IL-17-biased γδ T cells in healthy humans are rare, this review also comprehensively reports all the pathological situations where IL-17-producing human γδ T cells have been reported. Bonefeld and colleagues also elaborate on IL-17-γδ T cells with a special focus on inflammatory skin diseases.[9] Diverse mouse models are presented, such as allergic contact dermatitis, psoriasis, and atopic dermatitis, to discuss the inflammatory or anti-inflammatory functions of different subsets of γδ T cells in these pathologies. The authors develop interesting parallels to the results reported in the same pathologies in human patients. They also expose the role of epithelial cell molecules important for γδ T cell development that are also expressed under inflammatory conditions in the epidermis. Edelblum and colleagues also examine the role of γδ T cells at barrier surfaces. They conclude that epithelial tissues, especially mucosa, are the favorite location of γδ T cells both in mice and humans.[3] Local enrichment at these sites is fundamental for γδ T cell immune surveillance and their functions in tissue repair and barrier protection. Edelbum's team has produced seminal contributions on the visualization of γδ T cell motility within intestinal epithelium using state-of-art live imag-ing technologies (→see cover picture). They discuss in this issue the importance to define the specific migratory behaviors of γδ T cells within different tissue microenvironments including skin, reproduc-tive tract, lung, and intestines, a mean to understand how these cells scan the presence of pathogens or antigens to fulfill their surveil-lance purpose.

5 LIGAND RECOGNITION BY γδ TCR

Antigens recognized by γδ T cells are far from being comprehensively described but the progresses made during the last decade provided unexpected clues in our understanding of how and where γδ T cells are activated. Several papers in this issue debate mechanistic models of antigen recognition by Vγ9Vδ2 T cells. This preponderant popula-tion of semi-invariant γδ T cells in the human blood has long been known to recognize, in a TCR-dependent manner, small phosphorylated molecules that are metabolic compounds of the isoprenoid pathway and called "phosphoantigens".

The small size of these compounds made it most unlikely that a direct engagement of the TCR by pAg may activate Vg9Vd2 cells. Thus, the existence of a putative pAg presenting molecule has long been evoked without any evidence. A major breakthrough came in 2012, when Daniel Olive's team generated the 20.1 mAb directed against the butyrophilin family member BTN3A1 and Emanuel Scotet's team demonstrate that 20.1 mAb potently activates Vγ9Vδ2 T cells within PBMC. BTN3A1 expression was then shown to be necessary for Vγ9Vδ2 T cell activation by cells treated with nitrogen-containing bisphosphonates (N-BPs)-drugs blocking the isoprenoid pathway leading to the accumulation of pAg in treated cells. As described in detail in their contribution to this issue, Adams and colleagues have convincingly demonstrated through biochemical and struc-tural analyses that pAgs bind to the B30.2 intracellular domain of BTN3A1, eliciting conformational changes probably involved in the activation of Vγ9Vδ2 T cells.11 However, no indication that BTN3A1 can bind to Vγ9Vδ2 TCR has been provided so far, and the issue of the direct ligand of Vγ9Vδ2 TCR remained unre-solved for a long time. Nonetheless, early 2020 another member of the family, BTN2A1, came into the picture, resolving part of the enigma. Concomitantly but using completely different demon-strations, Godfrey and colleagues and Herrmann and colleagues recently revealed that BTN2A1 is able to directly bind the Vγ9 domain of Vγ9Vδ2 TCR and to associate with BTN3A1 on cell sur-face.[12,4] Both teams, as well as Willcox and colleagues who also contributed to this observation, thoroughly explain the rational used for their demonstrations in their respective papers published in this special issue.[13] Vγ9Vδ2 are not the only γδ T cell subset whose mode of recognition for antigens has been progressively deciphered during the last years. Growing interest developed during the recent years on the so far rather neglected other subsets of γδ T cells in the human system: the so-called non-Vγ9Vδ2 γδ T cells. These cells (mainly comprising $V\delta1^+$ T cells but also $V\delta3^+$ T cells) preferentially localize in tissues such as digestive or respiratory epithelia and liver. They can express any type of Vγ chain, including Vγ9, and a much more diverse TCR repertoire than Vγ9Vδ2 T cells. These cells have been involved in the response to viral infections, especially Cytomegalovirus (CMV) infection. The repertoire of the antigens they recognize is also deemed highly diverse. Adams and colleagues provide a comprehensive re-view on the MHC, MHC-like and non-MHC TCR ligands that have been reported as recognized by Vδ1 T cells.[11] A special emphasis is made on CD1d that is recognized as a lipid presenting molecule by a subset of Vδ1 T cells and on all the structural features associated with CD1d recognition by several Vδ1 TCRs.

6 RESPONSE OF γδ T CELL S TO INFECTIONS

One important issue that remains to be resolved regards the role of these ligands in a natural response of non-Vγ9Vδ2 γδ T cells. Most of the proposed ligand, such as CD1d, are recognized by γδ T cells that have not been involved in response to pathogen nor in pathological situation. As presented by Déchanet-Merville and colleagues the TCR of γδ T cell clones responding to CMV can recognize “self” molecules as native antigens either-for example, EPCR-in the context of a multi-molecular complex with inducible co-stimulatory ligands or-for example, Annexin A2-after stress-induced translocation to cell surface. Given the implication of γδ T cells in the control of CMV, both in human and mice, this review also discusses this infectious context as a relevant setting to understand a natural response of γδ T cells but also to harness γδ T cells toward a better monitoring of patients and toward anti-infectious T cell-based immunotherapy. The role of γδ T cells in infections is also the topic developed by Chen and colleagues who pioneered the investigation of Vγ9Vδ2 T cell responses to infections in primate models.[10] In this edition, they discuss their work on the rapid and protective action of adoptively transferred Vγ9Vδ2 T cells against *Mycobacterium tuberculosis*. They also present new results on Vγ9Vδ2 T response to the vaccination using an attenuated live *Listeria monocytogenes* vector and associa-tion with a better protection against *M tuberculosis*.

7 γδ T CELL S AND CANCER

γδ T cells have been found to infiltrate many different types of tu-mors in human cancers as well as in cancer models in mice. Their implication in the immune response to tumors has been the subject of many clinical studies and papers. γδ T cells have been associated with either bad or good prognosis depending on the cancer type and on γδ T cell profile-particularly their propensity to pro-duce either IL-17 or IFNγ. Our understanding of their function in cancer immunosurveillance and of how different tumor resistance mechanisms and immunosuppressive environments can affect γδ T cells’ anti-tumor activity still need to be improved. These are critical notions to be consolidated if one wants to exploit γδ T cells in cancer therapy. Five reviews develop these issues. After a description of gut γδ T cell development and functions, Coffelt and colleagues focus on colorectal cancer to depict our current knowledge about the anti-tumoral or pro-tumoral role of γδ T cells in this disease and the differences reported between human tumors and mouse models of colorectal cancer.16 Dieli and colleagues give a detailed presentation of the studies that have analyzed γδ T cell presence and phenotype in human tumors through various approaches (immunohistochemistry, flow cytometry, bulk or single-cell RNA sequencing).[17]

They note a tendency for a positive correlation between favorable prognosis and high frequencies of Vδ1 T cells or of IFNγ-producing Vδ2 T cells among tumor-infiltrating cells. Wesch and colleagues put emphasis on four negative regulators of γδ T cell activities: COX-2, IDO-1/2, TRAIL-R4 and galectins produced in tumors.[18] They propose new perspectives for the combination of different γδ T cell-based therapies with inhibitors targeting these immunosuppressive pathways. Two papers discuss new developments in the use of Vγ9Vδ2 T cells in adoptive cell therapy. Tanaka and colleagues list and discuss the different activities of pyrophosphonates, N-BPs or N-BP pro-drugs, in the expansion of Vγ9Vδ2 T cells for adoptive cancer cell therapy, and the interest to combine such therapy with check point inhibitors or cytokines as IL18.[19] Scotet and colleagues provide an overview of the different strategies that can be implemented to use Vγ9Vδ2 T cells in cell therapy.[20] They discuss the tumor microenvironment-related obstacles that have to be circumvented to improve Vγ9Vδ2 T cell targeting to the tumor and anti-tumor potential, and how genetic engineering could provide solutions toward that goal.

8 ADAPTIVE-LIKE VERSUS INNATE-LIKE ATTRIBUTES OF γδ T CELL S

While γδ T cells have long been classified within the innate-like lymphocytes or as intermediates between innate and adaptive immunity, growing and compelling evidences support their implication in adaptive immune responses and long-term protection. In humans, innate-like Vγ9Vδ2 T cells enriched in peripheral blood and responding to pAg can be distinguished from the adaptive-like non-Vγ9Vδ2 γδ T cells enriched in tissues. Likewise, preprogrammed innate-like mouse T cells expressing invariant TCRs (eg, DETC, Vγ6Vδ1T cells) can be opposed to the adaptive-like polyclonal Vγ4 and Vγ1 T cells generated during adult life. Using analysis of γδ T cell development, Vermijlen and colleagues clarify the role of innate-like γδ T cells in early life particularly regarding the defense against pathogens. Upon aging, γδ T cells become more adaptive-like with a more diversified repertoire and functions. They also reveal shared mechanisms to generate innate versus adaptive-like γδ T cells in mice and human. As developed by Déchanet-Merville and colleagues, infection by CMV has been reported as a prototypic situation for an adaptive response of γδ T cells, reported both in human and in mice, based on transcriptomic and phenotypic features, clonal expansion, and better response during reinfection when compared to primary infection. Interestingly, Herrmann and colleagues also dispute in their review the idea that monoclonal or oligoclonal expansion, generally considered as a hallmark of adaptive immune response, is necessarily the consequence of TCR-mediated antigen recognition.[4] Viewed from a molecular angle, Willcox and colleagues comment on the capacity of γδ TCRs to recognize constitutively expressed BTN/BTNL family members through germline-encoded regions of particular TCR Vγ regions, as opposed to the recognition of di-verse, mostly stress-induced but non-processed, antigens through the CDR3 regions of the γδTCR.13 It is not yet clear if and what specific functional activities are associated with each type of rec-ognition; however, these authors propose that these two distinct layers of γδ TCR reactivity discriminate innate-like from adaptive-like biology.

9 CONCLUSION

Together, this volume establishes an important update on the current directions of basic and translational research in the field of γδ T cells. Therefore, we hope that the comprised reviews will contribute to inspire future activities to harness the power of γδ T cells in novel therapeutic strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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