Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Antigen-specific γδ T cells contribute to cytomegalovirus control after stem cell transplantation

Immo Prinz[1,2] and Christian Koenecke[1,3]

γδ T cells support the immunological control of viral infections, in particular during cytomegalovirus (CMV) reactivation in immunocompromised patients after allogeneic hematopoietic stem cell transplantation. It is unclear how γδ T cells sense CMV-infection and whether this involves specific T cell receptor (TCR)-ligand interaction. Here we summarize recent findings that revealed an adaptive-like anti-CMV immune response of γδ T cells, characterized by acquisition of effector functions and long-lasting clonal expansion. We propose that rather CMV-induced self-antigen than viral antigens trigger γδ TCRs during CMV reactivation. Given that the TCRs of CMV-activated γδ T cells are often cross-reactive to tumor cells, these findings pinpoint γδ T cells and their γδ TCRs as attractive multipurpose tools for antiviral and antitumor therapy.

Addresses
[1] Institute of Immunology, Hannover Medical School (MHH), Germany
[2] Institute of Systems Immunology, University Medical Center Hamburg-Eppendorf, Germany
[3] Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, MHH, Germany

Corresponding author: Prinz, Immo (i.prinz@uke.de)

Current Opinion in Immunology 2023, **82**:102303

This review comes from a themed issue on **Chronic Infections**

Edited by **Thomas Mertens**

For complete overview of the section, please refer to the article collection, "Chronic Infections (2023)"

Available online 20 March 2023

https://doi.org/10.1016/j.coi.2023.102303

Introduction

Despite prophylactic or preemptive pharmacological treatment, cytomegalovirus (CMV) seropositivity and reactivation of remain associated with increased morbidity and mortality after allogeneic hematopoietic stem cell transplantation (alloHSCT) [1,2]. In healthy individuals, control of latent CMV infection depends on several layers of immune cells, in particular the lymphocyte lineages natural killer (NK) cells, αβ T cells, and γδ T cells. As recently discussed elsewhere [3,4], rapidly expanding donor NK cells support the control of CMV reactivation early after alloHSCT and may at the same time contribute to graft-versus-leukemia reactions. In contrast, the reconstitution of potent antiviral $CD4^+$ and $CD8^+$ αβ T cells takes several weeks to months and also depends on the CMV serostatus of the alloHSCT donor and leaves the patients susceptible to CMV reactivation or even CMV disease [5].

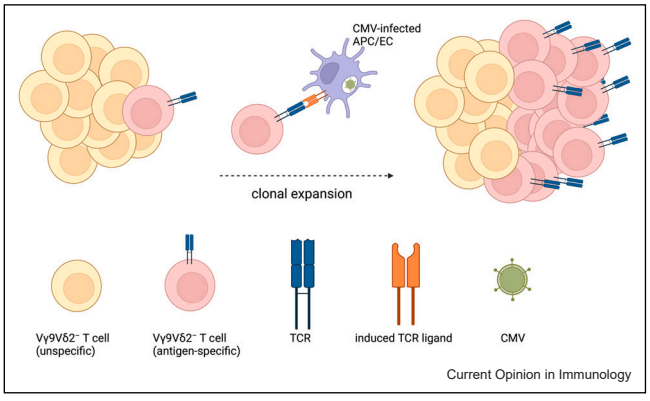
In this review, we report interdisciplinary work performed within a DFG-funded research consortium CRC900 investigating microbial persistence and its control in chronic infections. We focus on studies investigating the activation of γδ T cells in the context of CMV reactivation after alloHSCT, their potential γδ T cell receptors (TCR) ligands, and discuss their contribution to CMV control.

Main

Human γδ T cells come in two flavors, one is more innate and one is more adaptive. The presumably more innate γδ T cells uniformly use a canonical combination of Vγ9 and Vδ2 to form their very similar (semi-invariant) $Vγ9Vδ2^+$ TCR, while the potentially more adaptive γδ T cells display highly diverse TCRs composed of other Vγ and Vδ rearrangements [6,7]. The TCRs of the latter subset comprise mainly $Vδ1^+$, but also $Vδ3^+$ and other Vδ chains, as well as TCRs in which a Vδ2 chain pairs with other Vγ chains than the canonical Vγ9-JP chain. Those γδ T cells are thus collectively summarized as 'non-$Vγ9Vδ2^+$ T cells' or '$Vγ9Vδ2^-$ T cells' [8]. Over the past few years, $Vγ9Vδ2^-$ T cells were recognized to be 'adaptive' or 'adaptive-like' γδ T cells because they showed clonal expansions in response to viral infection and other immunogenic or inflammatory stimuli [9,10] (Figure 1).

In 2014, the starting point of our studies within the CRC900 consortium was the seminal observation that γδ T cells using $Vδ1^+$ TCRs were expanding in response to CMV infection in patients after kidney transplantation [11,12]. At the same time, there was solid evidence that a high frequency of γδ T cells would be beneficial for the disease-free and overall survival in patients after alloHSCT for leukemia [13–15]. This could be explained in part by the surprising finding that γδ T cells elicited by CMV reactivation cross-recognized CMV-infected and leukemia cells [16]. However, it was unclear whether $Vδ1^+$ T cells and other $Vγ9Vδ2^-$ T cells responded to CMV infection and leukemia via innate receptors such as NKG2D, similar to NK cells, or whether their

Figure 1



CMV-infection leads to 'adaptive' or 'adaptive-like' expansion of individual Vγ9Vδ2$^-$ T cell clones. Infection with CMV can induce the upregulation and surface expression of stress-induced TCR ligands such as MHC class II on antigen-presenting cells or endothelial cells (APC/EC, middle). These induced ligands can in turn activate certain Vγ9Vδ2$^-$ T cells (from a polyclonal pool of Vδ1$^+$ and Vδ3$^+$ T cells) that express a matching specific TCR, leading to clonal expansion of this γδ T cell clone. Created with BioRender.com.

individual TCR were triggered in some sort of adaptive T cell response.

Therefore, we set up a prospective patient cohort study to investigate how the human γδ T cell pool is regenerated after alloHSCT and to investigate how γδ T cells respond in patients after alloHSCT with and without episodes of CMV reactivation [9]. At the same time, we established an RNA-based bulk γδ TCR sequencing approach, which allowed us to monitor the dynamics of individual γδ T cell clones after alloHSCT based on the abundance of individual γδ TCR chains.

As a control group, we analyzed healthy adults and found that repertoires of the γδ TCRs encoding genes, *TRG* and *TRD*, in the peripheral blood were surprisingly stable over time. While a large fraction of human *TRG* repertoires consisted of 'public' TCR sequences that were identical in many individuals [17], TCR δ chain repertoires were largely 'private', meaning that individual *TRD* rearrangements are rarely shared between any two individuals [9]. Furthermore, we could later show that microbial exposure drove a polyclonal expansion of the more innate Vγ9Vδ2$^+$ T cells immediately after birth [18].

After alloHSCT, γδ T cells were quickly reconstituted, when patients had no complications, such as severe infection or graft-versus-host disease. However, they had profoundly altered TCR repertoires, which were dominated by stably expanded innate Vγ9Vδ2$^+$ T cell clones. In patients with CMV reactivation, we always observed a pronounced expansion of presumably virus-reactive Vδ1$^+$ and Vδ3$^+$ γδ T cells, in line with previous findings [19]. However, it was unclear whether a broad variety of different Vδ1$^+$ T cells and other non-Vγ9Vδ2$^+$ T cells were responding to CMV infection in an innate-like mode, similar to NK cells, or whether their expansion was driven by a few individual γδ TCR and led to an adaptive-like clonal T cell response, similar to CD8$^+$ αβ T cells. Indeed, in all alloHSCT patients with CMV reactivation, we observed a clonal proliferation of presumably virus-reactive Vγ9Vδ2$^-$ T cells [9]. This was in line with a concomitant study reporting that TCR repertoires of Vδ1$^+$ T cells in adult humans focused on a few high-frequency clonotypes, likely as a consequence of clonal responses to viral infection [10]. These data and other studies describing CMV-induced clonal immune responses of Vγ9Vδ2$^-$ T cells [20–23] collectively provided a strong argument for an adaptive-like anti-CMV immune response of γδ T cells, which might also extend to responses to other herpes viruses [24] and other infectious diseases including malaria [25,26]. Of note, immune responses of specific γδ T cell subsets were also reported to protect mice from MCMV infections [27,28].

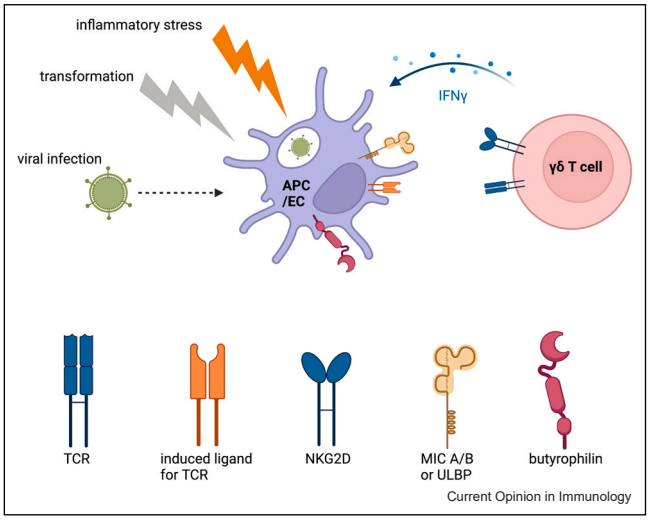
Until 2019, all studies monitoring population dynamics of γδ T cells, for example, those that identified clonal expansions of CMV-reactive γδ T cells [9], relied on bulk amplicon TCR sequencing of the rearranged *TRG* and *TRD* genes. Only recent technical advances in single-cell biology made it possible to link individual γδ T cell clones to pathways to their differentiation into effector T cells [29–33]. By combining scRNA-seq and scTCR-seq, these studies underlined the distinct differentiation trajectories of the more innate Vγ9Vδ2$^+$ T cells versus the more adaptive Vγ9Vδ2$^-$ T cells. Also, it is now feasible to follow the anti-CMV immune response of individual γδ T cell clones based on their unique TCR before, during, and after CMV reactivation in our longitudinal alloHSCT patient cohorts. This will further elucidate how far the TCR will instruct the differentiation of clonal T cells into effector and eventually long-lived memory T cells. This prospect of new unparalleled data is exciting, as to date only very few studies could directly observe T cell responses before, during, and after human herpesvirus infection [34].

Deseke et al. used combined single-cell γδ TCR-sequencing and single-cell RNA-sequencing to identify the exact γδ TCR and to define a cytotoxic effector T cell phenotype of those γδ T cells that expanded in response to CMV in alloHSCT patients [35]. In order to judge whether the observed expansions of individual clones were actually driven by TCR-dependent signals, we need information about the cognate γδ TCR ligands recognized by the expanding γδ T cells. So far, specific ligands were described for only few γδ TCR from Vγ9Vδ2$^-$ T cells [36]. Deseke et al. therefore used soluble γδ TCR staining of recognized target tumor cells

and applied CRISPR/Cas9-mediated screening to identify HLA-DRA, RFXAP, RFX5, and CIITA as required determinants for target cell recognition of a CMV-induced Vδ1$^+$ TCR. Further biochemical characterization using surface plasmon resonance assays revealed a direct interaction of this Vδ1$^+$ TCR with the MHC-II complex HLA-DR [35]. Since MHC class II molecules and in particular HLA-DR are directly upregulated by the inflammatory cytokine interferon-γ, these results suggested that this Vδ1$^+$ T cell clone was activated by an infection-induced self-antigen, which acts as a 'danger-signal'. Moreover, identifying HLA-DR as a ligand was unexpected but retrospectively not too surprising, because MHC class II molecules were among the first reported γδ TCR ligands [37,38]. Interestingly, also other groups have recently identified several additional examples of Vδ1$^+$ and Vδ3$^+$ TCRs that were specifically recognizing MHC and MHC-related molecules [39–44].

It is now clear that CMV-encoded antigen is not directly driving CMV-induced γδ T cell expansion, but rather some infection-induced self-antigen. So, is antigen-specific TCR signaling necessary and sufficient to drive clonal expansion of Vδ1$^+$ and Vδ3$^+$ T cells? Notably, recent evidence suggested that NK cells, which don't have any TCR, also mount some form of peptide antigen-specific immune responses [45]. Furthermore, an elegant recent study leveraged somatic mitochondrial DNA mutations as endogenous barcodes to show that clonal expansion and persistence of NK lymphocytes can be TCR-independent [46]. Thus, at this point, it is still possible that TCR-independent stimuli such as via NKG2D and NKG2C [47,48] may also contribute essentially to inducing a strong clonal proliferation of those Vδ1$^+$ and Vδ3$^+$ γδ T cells that are just at the right time in the right place during CMV reactivation. Figure 2 summarizes the signals that infected, stressed or transformed cells may upregulate to stimulate γδ T cells, which in turn can stimulate epithelial or professional antigen-presenting cells by secretion of inflammatory cytokines such as IFN-γ. Future studies should focus on deconvoluting the effects of TCR-dependent and TCR-independent mechanisms. This could be done experimentally using signaling knock-out mice in experimental models for human CMV infection [27,28]. In the human system, one can utilize *in vitro* expansion protocols in the presence or absence of mAb that block or stimulate the γδ TCR and/or innate activating receptors such as NKG2D [49]. In this context, it is clear that a mix of cytokines and antibodies recognizing pan-γδ TCR can lead to a polyclonal activation and expansion of cytotoxic Vδ1$^+$ T cells with antitumor properties [50,51]. Nevertheless, TCR recognition of stress-induced self-ligands on epithelial or professional antigen-presenting cells may be TCR-dependent but still regarded as an innate-like mechanisms of effector T cell activation.

Figure 2



γδ T cells act at the frontier in between innate and adaptive immunity. Next to specific ligands engaging the γδ TCR, CMV-infection, inflammatory stress, and cell transformation can induce the expression of stress-induced ligands such as MIC A/B, ULBP on antigen-presenting cells or endothelial cells (APC/EC). These ligands may contribute to activate γδ T cells via cell stress-sensing NKG2D receptor, leading to clonal expansion and secretion of inflammatory cytokines such as IFN-γ. Created with BioRender.com.

Conclusions

In conclusion, the studies discussed here collectively suggest that CMV infection induces a sustained adaptation of γδ T cells, which confer protective antiviral immunity. In the setting of alloHSCT, a long-lasting expansion of CMV-induced effector γδ T cells provides a dual benefit, namely antiviral and antitumor immune surveillance. Therefore, γδ T cell responses should be leveraged for the treatment of CMV reactivation in the setting of alloHSCT and solid organ transplantation. This might be achieved by depleting only αβ T cells and B cells from the stem cell graft while NK cells and γδ T cells remain to serve as an innate bridge [52–54], or by therapeutic interventions to overcome the negative impact of immunosuppressive drugs on γδ T cell effector function [55,56]. Since γδ T cells do not induce GvHD, other suitable strategies could be based on adaptive therapies using *ex vivo* expanded allogeneic γδ T cells or γδ CAR-T cells [57].

Data Availability

No data were used for the research described in the article.

Conflict of interest statement

Both authors declare no conflict of interest.

Acknowledgements

This work was supported by Grants from the Deutsche Forschungsgemeinschaft (DFG), Germany, to IP and CK: DFG-CRC900/B8 and DFG-CRC900/Z1, 2014–2022, project number 158989968.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, Green JS, Saad A, Antin JH, Savani BN, *et al.*: **Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis**. *Blood* 2016, **127**:2427-2438.

2. Ljungman P, Brand R, Hoek J, de la Camara R, Cordonnier C, Einsele H, Styczynski J, Ward KN, Cesaro S, *et al.*, Infectious Diseases Working Party of the European Group for B: **Donor cytomegalovirus status influences the outcome of allogeneic stem cell transplant: a study by the European group for blood and marrow transplantation**. *Clin Infect Dis* 2014, **59**:473-481.

3. Bigley AB, Baker FL, Simpson RJ: **Cytomegalovirus: an unlikely ally in the fight against blood cancers?** *Clin Exp Immunol* 2018, **193**:265-274.

4. Litjens NHR, van der Wagen L, Kuball J, Kwekkeboom J: **Potential beneficial effects of cytomegalovirus infection after transplantation**. *Front Immunol* 2018, **9**:389.

5. Degli-Esposti MA, Hill GR: **Immune control of cytomegalovirus reactivation in stem cell transplantation**. *Blood* 2022, **139**:1277-1288.

6. McVay LD, Carding SR, Bottomly K, Hayday AC: **Regulated expression and structure of T cell receptor gamma/delta transcripts in human thymic ontogeny**. *EMBO J* 1991, **10**:83-91.

7. Vermijlen D, Prinz I: **Ontogeny of innate T lymphocytes — some innate lymphocytes are more innate than others**. *Front Immunol* 2014, **5**:486.

8. Deseke M, Prinz I: **Ligand recognition by the gammadelta TCR and discrimination between homeostasis and stress conditions**. *Cell Mol Immunol* 2020, **17**:914-924.

9. •• Ravens S, Schultze-Florey C, Raha S, Sandrock I, Drenker M, Oberdorfer L, Reinhardt A, Ravens I, Beck M, Geffers R, *et al.*: **Human gammadelta T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection**. *Nat Immunol* 2017, **18**:393-401.
This work performed within CRC900 used bulk and single cell γδ TCR sequencing of a prospective patient study to show pronounced clonal expansion of Vδ1+ and Vδ3+ γδ T cells after CMV reactivation.

10. • Davey MS, Willcox CR, Joyce SP, Ladell K, Kasatskaya SA, McLaren JE, Hunter S, Salim M, Mohammed F, Price DA, *et al.*: **Clonal selection in the human Vdelta1 T cell repertoire indicates gammadelta TCR-dependent adaptive immune surveillance**. *Nat Commun* 2017, **8**:14760.
This study used bulk γδ TCR sequencing to identify highly expanded Vδ1+ clones in the peripheral blood of healthy donors.

11. Dechanet J, Merville P, Berge F, Bone-Mane G, Taupin JL, Michel P, Joly P, Bonneville M, Potaux L, Moreau JF: **Major expansion of gammadelta T lymphocytes following cytomegalovirus infection in kidney allograft recipients**. *J Infect Dis* 1999, **179**:1-8.

12. Dechanet J, Merville P, Lim A, Retiere C, Pitard V, Lafarge X, Michelson S, Meric C, Hallet MM, Kourilsky P, *et al.*: **Implication of gammadelta T cells in the human immune response to cytomegalovirus**. *J Clin Investig* 1999, **103**:1437-1449.

13. Lamb LS Jr., Henslee-Downey PJ, Parrish RS, Godder K, Thompson J, Lee C, Gee AP: **Increased frequency of TCR gamma delta + T cells in disease-free survivors following T cell-depleted, partially mismatched, related donor bone marrow transplantation for leukemia**. *J Hematother* 1996, **5**:503-509.

14. Godder KT, Henslee-Downey PJ, Mehta J, Park BS, Chiang KY, Abhyankar S, Lamb LS: **Long term disease-free survival in acute leukemia patients recovering with increased gammadelta T cells after partially mismatched related donor bone marrow transplantation**. *Bone Marrow Transpl* 2007, **39**:751-757.

15. Klyuchnikov E, Badbaran A, Massoud R, Fritsche-Friedland U, Janson D, Ayuk F, Wolschke C, Bacher U, Kroger N: **Enhanced immune reconstitution of gammadelta T cells after allogeneic stem cell transplantation overcomes the Negative impact of pretransplantation minimal residual disease-positive status in patients with acute myelogenous leukemia**. *Transpl Cell Ther* 2021, **27**:841-850.

16. Scheper W, van Dorp S, Kersting S, Pietersma F, Lindemans C, Hol S, Heijhuurs S, Sebestyen Z, Grunder C, Marcu-Malina V, *et al.*: **gammadeltaT cells elicited by CMV reactivation after allo-SCT cross-recognize CMV and leukemia**. *Leukemia* 2013, **27**:1328-1338.

17. Sherwood AM, Desmarais C, Livingston RJ, Andriesen J, Haussler M, Carlson CS, Robins H: **Deep sequencing of the human TCRgamma and TCRbeta repertoires suggests that TCRbeta rearranges after alphabeta and gammadelta T cell commitment**. *Sci Transl Med* 2011, **3**:90ra61.

18. Ravens S, Fichtner AS, Willers M, Torkornoo D, Pirr S, Schoning J, Deseke M, Sandrock I, Bubke A, Wilharm A, *et al.*: **Microbial exposure drives polyclonal expansion of innate gammadelta T cells immediately after birth**. *Proc Natl Acad Sci USA* 2020, **117**:18649-18660.

19. Knight A, Madrigal AJ, Grace S, Sivakumaran J, Kottaridis P, Mackinnon S, Travers PJ, Lowdell MW: **The role of Vdelta2-negative gammadelta T cells during cytomegalovirus reactivation in recipients of allogeneic stem cell transplantation**. *Blood* 2010, **116**:2164-2172.

20. Tuengel J, Ranchal S, Maslova A, Aulakh G, Papadopoulou M, Drissler S, Cai B, Mohsenzadeh-Green C, Soudeyns H, Mostafavi S, *et al.*: **Characterization of adaptive-like gammadelta T cells in Ugandan infants during primary cytomegalovirus infection**. *Viruses* 2021, **13**.

21. Vermijlen D, Brouwer M, Donner C, Liesnard C, Tackoen M, Van Rysselberge M, Twite N, Goldman M, Marchant A, Willems F: **Human cytomegalovirus elicits fetal gammadelta T cell responses in utero**. *J Exp Med* 2010, **207**:807-821.

22. Kaminski H, Menard C, El Hayani B, Adjibabi AN, Marseres G, Courant M, Zouine A, Pitard V, Garrigue I, Burrel S, *et al.*: **Characterization of a unique gammadelta T-cell subset as a specific marker of cytomegalovirus infection severity**. *J Infect Dis* 2021, **223**:655-666.

23. Gaballa A, Arruda LCM, Radestad E, Uhlin M: **CD8(+) gammadelta T cells are more frequent in CMV seropositive bone marrow grafts and display phenotype of an adaptive immune response**. *Stem Cells Int* 2019, **2019**:6348060.

24. Martini F, Champagne E: **The contribution of human Herpes viruses to gammadelta T cell mobilisation in co-infections**. *Viruses* 2021, **13**.

25. Leon-Lara X, Yang T, Fichtner AS, Bruni E, von Kaisenberg C, Eiz-Vesper B, Dodoo D, Adu B, Ravens S: **Evidence for an adult-like Type 1-immunity phenotype of Vdelta1, Vdelta2 and Vdelta3 T Cells in Ghanaian children with repeated exposure to malaria**. *Front Immunol* 2022, **13**:807765.

26. von Borstel A, Chevour P, Arsovski D, Krol JMM, Howson LJ, Berry AA, Day CL, Ogongo P, Ernst JD, Nomicos EYH, *et al.*: **Repeated *Plasmodium falciparum* infection in humans drives the clonal expansion of an adaptive gammadelta T cell repertoire**. *Sci Transl Med* 2021, **13**:eabe7430.

27. Khairallah C, Netzer S, Villacreces A, Juzan M, Rousseau B, Dulanto S, Giese A, Costet P, Praloran V, Moreau JF, *et al.*: **gammadelta T cells confer protection against murine cytomegalovirus (MCMV)**. *PLoS Pathog* 2015, **11**:e1004702.

28. Sell S, Dietz M, Schneider A, Holtappels R, Mach M, Winkler TH: **Control of murine cytomegalovirus infection by gammadelta T cells**. *PLoS Pathog* 2015, **11**:e1004481.

29. Pizzolato G, Kaminski H, Tosolini M, Franchini DM, Pont F, Martins F, Valle C, Labourdette D, Cadot S, Quillet-Mary A, *et al.*: **Single-cell RNA sequencing unveils the shared and the distinct cytotoxic hallmarks of human TCRVdelta1 and TCRVdelta2 gammadelta T lymphocytes**. *Proc Natl Acad Sci USA* 2019, **116**:11906-11915.

30. • Tan L, Fichtner AS, Bruni E, Odak I, Sandrock I, Bubke A, Borchers A, Schultze-Florey C, Koenecke C, Forster R, *et al.*: **A fetal wave of human type 3 effector gammadelta cells with restricted TCR diversity persists into adulthood**. *Sci Immunol* 2021, **6**:eabf0125.
This work performed within CRC900 combined droplet-based single cell RNA sequencing and single cell γδ TCR sequencing of cord blood and peripheral blood to identify type- 3 effector γδ T cells that appear already very early in life.

31. Tan L, Sandrock I, Odak I, Aizenbud Y, Wilharm A, Barros-Martins J, Tabib Y, Borchers A, Amado T, Gangoda L, *et al.*: **Single-cell transcriptomics identifies the adaptation of Scart1(+) Vgamma6(+) T cells to skin residency as activated effector cells**. *Cell Rep* 2019, **27**:3657-3671 e3654.

32. Sagar, Pokrovskii M, Herman JS, Naik S, Sock E, Zeis P, Lausch U, Wegner M, Tanriver Y, Littman DR, *et al.*: **Deciphering the regulatory landscape of fetal and adult gammadelta T-cell development at single-cell resolution**. *EMBO J* 2020, **39**:e104159.

33. •• Sanchez Sanchez G, Papadopoulou M, Azouz A, Tafesse Y, Mishra A, Chan JKY, Fan Y, Verdebout I, Porco S, Libert F, *et al.*: **Identification of distinct functional thymic programming of fetal and pediatric human gammadelta thymocytes via single-cell analysis**. *Nat Commun* 2022, **13**:5842.
New and technically sound evidence for intrathymic acquisition of effector functions by human γδ T cells.

34. Aslan N, Watkin LB, Gil A, Mishra R, Clark FG, Welsh RM, Ghersi D, Luzuriaga K, Selin LK: **Severity of acute infectious mononucleosis correlates with cross-reactive influenza CD8 T-cell receptor repertoires**. *mBio* 2017, **8**:e01841-17.

35. •• Deseke M, Rampoldi F, Sandrock I, Borst E, Boning H, Ssebyatika GL, Jurgens C, Pluckebaum N, Beck M, Hassan A, *et al.*: **A CMV-induced adaptive human Vdelta1+ gammadelta T cell clone recognizes HLA-DR**. *J Exp Med* 2022, **219**:e20212525.
This work performed within CRC900 used droplet-based single cell γδ TCR sequencing and CRISPR/Cas whole genome screening to identify HLA-DR as the cognate TCR ligand of a Vδ1+ γδ T cell clone that was expanded in a patient after CMV reactivation.

36. Willcox BE, Willcox CR: **gammadelta TCR ligands: the quest to solve a 500-million-year-old mystery**. *Nat Immunol* 2019, **20**:121-128.

37. Schild H, Chien YH: **The recognition of MHC molecules by gamma delta T cells**. *Behring Inst Mitt* 1994, **94**:113-123.

38. Schild H, Mavaddat N, Litzenberger C, Ehrich EW, Davis MM, Bluestone JA, Matis L, Draper RK, Chien YH: **The nature of major histocompatibility complex recognition by gamma delta T cells**. *Cell* 1994, **76**:29-37.

39. Benveniste PM, Roy S, Nakatsugawa M, Chen ELY, Nguyen L, Millar DG, Ohashi PS, Hirano N, Adams EJ, Zuniga-Pflucker JC: **Generation and molecular recognition of melanoma-associated antigen-specific human gammadelta T cells**. *Sci Immunol* 2018, **3**.

40. Roy S, Ly D, Castro CD, Li NS, Hawk AJ, Altman JD, Meredith SC, Piccirilli JA, Moody DB, Adams EJ: **Molecular analysis of lipid-reactive Vdelta1 gammadelta T cells identified by CD1c tetramers**. *J Immunol* 2016, **196**:1933-1942.

41. Le Nours J, Gherardin NA, Ramarathinam SH, Awad W, Wiede F, Gully BS, Khandokar Y, Praveena T, Wubben JM, Sandow JJ, *et al.*: **A class of gammadelta T cell receptors recognize the underside of the antigen-presenting molecule MR1**. *Science* 2019, **366**:1522-1527.

42. Uldrich AP, Le Nours J, Pellicci DG, Gherardin NA, McPherson KG, Lim RT, Patel O, Beddoe T, Gras S, Rossjohn J, *et al.*: **CD1d-lipid antigen recognition by the gammadelta TCR**. *Nat Immunol* 2013, **14**:1137-1145.

43. Wegrecki M, Ocampo TA, Gunasinghe SD, von Borstel A, Tin SY, Reijneveld JF, Cao TP, Gully BS, Le Nours J, Moody DB, *et al.*: **Atypical sideways recognition of CD1a by autoreactive gammadelta T cell receptors**. *Nat Commun* 2022, **13**:3872.

44. • Rice MT, von Borstel A, Chevour P, Awad W, Howson LJ, Littler DR, Gherardin NA, Le Nours J, Giles EM, Berry R, *et al.*: **Recognition of the antigen-presenting molecule MR1 by a Vdelta3(+) gammadelta T cell receptor**. *Proc Natl Acad Sci USA* 2021, **118**:e2110288118.
Important structural evidence for recognition of MHC-like MR-1 molecules by Vδ1+ and Vδ3+ γδ TCR.

45. Hammer Q, Ruckert T, Borst EM, Dunst J, Haubner A, Durek P, Heinrich F, Gasparoni G, Babic M, Tomic A, *et al.*: **Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells**. *Nat Immunol* 2018, **19**:453-463.

46. •• Ruckert T, Lareau CA, Mashreghi MF, Ludwig LS, Romagnani C: **Clonal expansion and epigenetic inheritance of long-lasting NK cell memory**. *Nat Immunol* 2022, **23**:1551-1563.
This elegant study used single cell analyses of somatic mitochondrial DNA mutations as endogenous barcodes to reveal clonal expansion of adaptive NK cells in HCMV+ individuals.

47. Liu R, Wu N, Gao H, Liang S, Yue K, Dong T, Dong X, Xu LP, Wang Y, Zhang XH, *et al.*: **Distinct activities of Vdelta1(+) T-cells upon different cytomegalovirus reactivation status after haematopoietic transplantation**. *Immunology* 2022, **167**:368-383.

48. Ishiyama K, Arakawa-Hoyt J, Aguilar OA, Damm I, Towfighi P, Sigdel T, Tamaki S, Babdor J, Spitzer MH, Reed EF, *et al.*: **Mass cytometry reveals single-cell kinetics of cytotoxic lymphocyte evolution in CMV-infected renal transplant patients**. *Proc Natl Acad Sci USA* 2022, **119**:e2116588119.

49. Dutta I, Postovit LM, Siegers GM: **Apoptosis induced via gamma delta T cell antigen receptor "Blocking" antibodies: a cautionary tale**. *Front Immunol* 2017, **8**:776.

50. Almeida AR, Correia DV, Fernandes-Platzgummer A, da Silva CL, da Silva MG, Anjos DR, Silva-Santos B: **Delta One T cells for immunotherapy of chronic lymphocytic leukemia: clinical-grade expansion/differentiation and preclinical proof of concept**. *Clin Cancer Res* 2016, **22**:5795-5804.

51. Di Lorenzo B, Simoes AE, Caiado F, Tieppo P, Correia DV, Carvalho T, da Silva MG, Dechanet-Merville J, Schumacher TN, Prinz I, *et al.*: **Broad cytotoxic targeting of acute myeloid leukemia by polyclonal Delta One T cells**. *Cancer Immunol Res* 2019, **7**:552-558.

52. Bethge WA, Eyrich M, Mielke S, Meisel R, Niederwieser D, Schlegel PG, Schulz A, Greil J, Bunjes D, Brecht A, *et al.*: **Results of a multicenter phase I/II trial of TCRalphabeta and CD19-depleted haploidentical hematopoietic stem cell transplantation for adult and pediatric patients**. *Bone Marrow Transpl* 2022, **57**:423-430.

53. Giardino S, Bagnasco F, Falco M, Miano M, Pierri F, Risso M, Terranova P, Di Martino D, Massaccesi E, Ricci M, *et al.*: **Haploidentical stem cell transplantation after TCR-alphabeta (+) and CD19(+) cells depletion in children with congenital non-malignant disease**. *Transpl Cell Ther* 2022, **28**:394 e391-394 e399.

54. Sperl D, Lang P, Benesch M, Bainschab A, Urban C, Wilfing R, Feuchtinger T, Doring M, Seitz C, Strenger V, *et al.*: **Immunological recovery following HLA-matched CD3+ TCR alphass+/CD19+ depleted hematopoietic stem cell transplantation in children**. *Pediatr Transpl* 2022, **26**:e14285.

55. • Kaminski H, Marseres G, Yared N, Nokin MJ, Pitard V, Zouine A, Garrigue I, Loizon S, Capone M, Gauthereau X, *et al.*: **mTOR inhibitors prevent CMV infection through the restoration of functional alphabeta and gammadelta T cells in kidney transplantation**. *J Am Soc Nephrol* 2022, **33**:121-137.
This work suggests that mTOR inhibitors a clever therapeutic strategy to improve natural T cell immune responses to CMV infection in immunocompromised patients.

56. Bestard O, Crespo E: **Disarming the old foe. Restoring T-cell immune function with mTor-inhibitors to tackle cytomegalovirus infection**. *J Am Soc Nephrol* 2022, **33**:6-8.

57. Morandi F, Yazdanifar M, Cocco C, Bertaina A, Airoldi I: **Engineering the bridge between innate and adaptive immunity for cancer immunotherapy: focus on gammadelta T and NK cells**. *Cells* 2020, **9**:1757.