REVIEW

Current progress in $\gamma\delta$ T-cell biology

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T lymphocytes bearing γ - and δ -chain T-cell receptor heterodimers are named $\gamma\delta$ T cells. Interestingly, $\gamma\delta$ and $\alpha\beta$ T cells share the same progenitors, and they undergo a fate decision in the thymus. Functional differentiation of $\gamma\delta$ T cells occurs both inside and outside the thymus. Antigen recognition of $\gamma\delta$ T-cell receptors is very unique, and the responses frequently exhibit innate characteristics. Nevertheless, peripheral $\gamma\delta$ T cells exert a number of effector and regulatory functions. $\gamma\delta$ T cells rapidly produce cytokines like interferon (IFN)- γ and IL-17 and promote inflammation, partly due to the inherent epigenetic and transcriptional programs, which facilitates a quick and extensive response. Moreover, $\gamma\delta$ T cells lyse target cells directly, and this is necessary for pathogen or tumor clearance. $\gamma\delta$ T cells can even serve as regulatory cells, and may contribute to immune suppression. Orchestration of $\gamma\delta$ T-cell and other immune cell interactions may be critical for host defense and immune regulation. Recently, $\gamma\delta$ T cells have been used for immunotherapy for infectious diseases and malignancy. In this review, we summarize the abstracts presented at the recent $\gamma\delta$ T cell Conference held from 19 to 21 May 2010, in Kiel, Germany (please see the website for details: <http://www.gammadelta-conference.uni-kiel.de/index.html>).

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INTRODUCTION

So far, two subsets of T cells have been defined: $\alpha\beta$ T cells and $\gamma\delta$ T cells. Peptide-major histocompatibility complex-specific T-cell responses are known to be achieved by $\alpha\beta$ T cells, while $\gamma\delta$ T-cell biology is much less studied. Mice without $\gamma\delta$ T cells are highly susceptible to diseases and tumor, suggesting an important role of $\gamma\delta$ T cells in defense. By current technology in cellular and molecular immunology, researchers revealed that $\gamma\delta$ T cells produce a series of cytokines in pathology, and the effector $\gamma\delta$ T cells play an indispensable role in pathogen elimination, immune regulation and autoimmunity. As a group of innate immune cells, $\gamma\delta$ T cells respond rapidly and expand efficiently when stimulated by antigens or cytokines. $\gamma\delta$ T cells may be a good target for modulation of immune responses in human diseases. In recent years, a lot of researchers focused on the $\gamma\delta$ T-cell development, function and therapeutic use.

DEVELOPMENT

Several factors that contribute to $\gamma\delta$ T-cell fate decisions have been identified, e.g. SOX13,¹ Id3,² T-cell receptor (TCR) signal strength, etc.^{3–10} However, mechanisms of functional differentiation of effector $\gamma\delta$ T cells are poorly understood.^{11–14} Key cytokines produced by $\gamma\delta$ T cells are interferon (IFN)- γ ,^{15–19} tumor-necrosis factor (TNF)- α ,^{20,21} and IL-17,^{22–26} and are critical for pathogen clearance, immune regulation and autoimmunity. Shibata and colleagues reported that the basic helix–loop–helix protein HES1 is specifically expressed in IL-17⁺ $\gamma\delta$ T cells and is required for the differentiation of naturally occurring IL-17⁺ $\gamma\delta$ T cells in the thymus. In addition, Powolny-Budnicka and colleagues

reported that RelA and RelB within different thymocyte subpopulations control IL-17 production by $\gamma\delta$ T cells and contribute to the host's ability to fight bacterial infections *in vivo*. CD27⁺ $\gamma\delta$ T cells produce IFN- γ while CD27[−] $\gamma\delta$ T cells produce IL-17.²⁷ However, the role and extent of $\gamma\delta$ TCR signaling, and how these signals cooperate with other thymic inputs to control CD27⁺ and CD27[−] $\gamma\delta$ T-cell development, remain unclear. Pang and colleagues reported that manipulating the strength of $\gamma\delta$ TCR signals can regulate CD27⁺ and CD27[−] $\gamma\delta$ T-cell development and produce IFN- γ and IL-17, respectively. Moreover, Michel and colleagues found that $\gamma\delta$ T-cell progenitors with the highest potential to produce IL-17 compose a small subset of cells characterized by high levels of receptor for a key thymic cytokine. Provision of this cytokine dramatically skews thymocytes towards IL-17 differentiation. Conversely, $\gamma\delta$ T cells with the highest potential to produce IFN- γ are promoted by engaging CD27.

Although the CD70–CD27 interaction was reported to be important for thymic differentiation of IFN- γ -producing $\gamma\delta$ T cells,²⁷ the role of this signal in the peripheral $\gamma\delta$ T cells remains unknown. Ribot and colleagues reported that CD27 is also required for peripheral expansion of the effector CD27⁺ $\gamma\delta$ T cells upon herpes and malaria infection in mice. Similarly, deBarros and colleagues in the same laboratory reported CD27 is expressed by ~80% of adult V γ 9 peripheral blood lymphocytes and by similar proportion of V γ 9⁺ thymocytes derived from paediatric thymic biopsies. CD27 signals promote the secretion of IFN- γ and TNF- α by human V γ 9V δ 2 $\gamma\delta$ T cells, but may not affect $\gamma\delta$ T-cell proliferation.

Because $\gamma\delta$ TCR itself is the only phenotypic marker for tracking thymocytes committed to the $\gamma\delta$ T-cell lineage, investigation of $\gamma\delta$

T-cell development has relied mainly on monitoring of the expression of $\gamma\delta$ TCR. Therefore, it has been impossible to identify $\gamma\delta$ T-cell precursors before they express $\gamma\delta$ TCR. Prinz and colleagues developed *TCRd-H2BEGFP* mice expressing a stable reporter gene 'knocked into' the *Tcrd* constant region gene.²⁸ Using the reporter mice, Ravens and colleagues explored the ontogeny of IL-17A-producing $\gamma\delta$ T cells in embryonic thymus. They found higher frequencies of IL-17A-producing $\gamma\delta$ T cells in *CCR7*^{-/-}, but lower ones in *CCR9*^{-/-} *TCRd-H2BEGFP* reporter mice, suggesting that signals from the thymic cortex may facilitate the development of IL-17A-producing $\gamma\delta$ T cells.

ANTIGENS AND COSTIMULATORY MOLECULES

To date, researchers have not fully elucidated the ligands for $\gamma\delta$ T cells, although several ligands of $\gamma\delta$ TCR have been identified.²⁹ Quite surprisingly, there are significant differences between mice and humans in the range of ligands recognized by the $\gamma\delta$ TCR. While phosphorylated small molecules that are intermediates of the microbial mevalonate metabolic pathway ('phosphoantigens') are prominent ligands for the human V γ 9V δ 2 T cells, such ligands are not recognized by murine $\gamma\delta$ T cells. Not taking into account such species differences, $\gamma\delta$ TCRs recognize T10/T22,³⁰⁻³³ CD1c,^{34,35} major histocompatibility complex class I-related chains A and B (MICA/B),³⁶ phosphoantigens³⁷ and ATP synthase-1/apolipoprotein A-1 complex.³⁸ Striegl and colleagues reported that CD1d is able to bind and present tetraacylated phospholipid cardiolipin to a subset of CL-responsive $\gamma\delta$ T cells that exist in the spleen and liver of healthy mice. Born and colleagues found that insulin-derived peptide B:9-23 is a ligand for murine $\gamma\delta$ TCRs. Although insulin peptide B:9-23 can also stimulate $\alpha\beta$ T cells, the $\gamma\delta$ T-cell response to insulin peptide B:9-23 does not require any accessory cells. Chain and colleagues are using recombinant soluble $\gamma\delta$ TCRs to find new ligands. Their laboratory (headed by Born) is currently focusing on identifying ligands for V γ 1V δ 6.3 and V γ 6V δ 1 TCRs. Detecting ligands of $\gamma\delta$ TCR multimers provides us with an opportunity to determine when, where and by whom $\gamma\delta$ TCR ligands are expressed.

It was reported that human $\gamma\delta$ TCRs recognize F1-ATPase and also the phosphoantigen isopentenyl pyrophosphate (IPP).^{37,38} Mookerjee-Basu and colleagues reported that F1-ATPase serves as an Ag-presenting molecule in this process. Immobilized F1 complexes can induce an intracellular calcium signal in IPP-specific V γ 9V δ 2 $\gamma\delta$ T cells in the presence of soluble IPP and in the absence of any cell-cell interactions.

NKG2D ligands are intriguing because of their multiplicity.³⁹ MICA was reported to activate NKG2D expressed on most human $\gamma\delta$ T cells,⁴⁰ Vantourout and colleagues studied the activation of $\gamma\delta$ T cells by MICA and the functional implications of its polymorphism. They observed broad diversity of the responses to different alleles of MICA.

Recently, Toll-like receptors were found to be expressed in $\gamma\delta$ T cells.²⁵ Here, Marischen and colleagues reported that human $\gamma\delta$ T cells express the Nod-like receptor NOD2 and thereby can react to its ligand muramyl dipeptide by increased IFN- γ secretion.

Although negative regulation of $\alpha\beta$ T cells has been widely studied, those of $\gamma\delta$ T cells are less well characterized. Specifically, cytotoxic T-lymphocyte antigen-4 and the immunoinhibitory receptor-programmed death-1 (PD-1) are major co-inhibitors of $\alpha\beta$ T cells and anti-cytotoxic T-lymphocyte antigen-4 and PD-1 monoclonal antibodies are used in clinical trials.⁴¹ Gertner-Dardenne and colleagues reported that PD-1 is readily expressed on resting V γ 9V δ 2 $\gamma\delta$ T cells and its expression was regulated during phosphoantigenic activation. Furthermore, a blockade of PD-1 resulted in increased

phosphoantigen-induced $\gamma\delta$ T-cell proliferation and Th1 cytokine secretion. Altogether, these data suggest that PD-1 is a major inhibitory functional receptor on V γ 9V δ 2 T cells and is also a potential therapeutic target for designing measures to modulate the $\gamma\delta$ T-cell response. Consistently, Beck and colleagues reported that the PD-1 pathway is a potentially important mechanism by which $\gamma\delta$ T cells are either functionally impaired or otherwise exhausted in tumor-bearing mice.

IMMUNE REGULATION

$\gamma\delta$ T cells are highly cytolytic lymphocytes that produce large amounts of proinflammatory cytokines during immune responses to multiple pathogens.^{42,43} However, how $\gamma\delta$ T-cell functions might be regulated remains unclear. Gonçalves-Sousa and colleagues reported that murine CD4⁺CD25⁺Foxp3⁺ regulatory T cells abolished key effector functions and proliferation of $\gamma\delta$ T cells both *in vitro* and *in vivo*. They further showed that suppression was dependent on cellular contact between regulatory and $\gamma\delta$ T cells, and was partially mediated by glucocorticoid-induced TNF receptor-related protein. These data revealed a novel mechanism by which $\gamma\delta$ T-cell function is regulated, and suggested that endogenous regulatory T cells may prevent the desired effects of $\gamma\delta$ T cell-based immunotherapies.

$\gamma\delta$ T cells play suppressive roles under some conditions.^{44,45} Otsuka and colleagues classified a fraction of $\gamma\delta$ T cells in the lymph nodes and all epidermal $\gamma\delta$ T cells in the skin, but not on dermal $\gamma\delta$ T cells in the steady state. These cells (IL-10⁺) extensively inhibited dendritic cell (DC)-dependent T-cell proliferation. Furthermore, they identified a specific marker of these regulatory $\gamma\delta$ T cells.

Gut intraepithelial lymphocytes constitute one of the largest T-cell compartments, and include large numbers of $\gamma\delta$ T cells.⁴⁶ Abeler-Dörner and colleagues described a novel means to establish viable, resting $\gamma\delta$ intraepithelial lymphocytes in culture for at least 3 weeks. They found activated intraepithelial lymphocytes induced the juxtaposed epithelial cells to substantially upregulate IL-6. The secretion of IL-6 is regulated by the epithelial surface molecule butyrophilin-like 1, which is specifically expressed by cells in the intestine. This network may jointly regulate antimicrobial responses, inflammation-associated carcinogenesis and food allergies.

FUNCTION IN INFECTIOUS DISEASES

$\gamma\delta$ T cells are important in virus and bacteria clearance.⁴⁷⁻⁵⁰ $\gamma\delta$ T cells may undergo a dysfunction during infection: Christopoulos and colleagues reported that, in patients with thymoma, expanded $\gamma\delta$ T cells bear a dysfunctional $\gamma\delta$ TCR that possesses the characteristics of altered stability and glycosylation, resulting in lowered reactivity to antigens. Furthermore, Meraviglia and colleagues claimed that *Mycobacterium tuberculosis*-infected DCs can only partially and ineffectively activate V γ 9V δ 2 T cells into a central memory type but not effector type.

While $\gamma\delta$ T cells are dysfunctional in infectious diseases, many substances can induce activation and expansion of them. Cimini and colleagues reported that IPH-1101 (i.e., bromohydrin pyrophosphate)-activated V γ 9V δ 2 T cells are able to mediate a direct antiviral activity and to improve adaptive long-lasting immune response in hepatitis C virus (HCV) patients after IFN- α treatment. Lin and colleagues found that as V γ 9V δ 2 T cells can be activated by (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), higher HMBPP-producing capacity of the causative pathogen predicts a better outcome in first-time episodes of bacterial peritonitis.

DCs, vaccine, microbial load, etc. are also involved in $\gamma\delta$ T-cell activation.^{23,25,51–54} Moens and colleagues showed that human neonatal DC-derived IL-23 combined with specific TCR signaling drives the generation of neonatal V γ 9V δ 2 T cells equipped with a range of cytotoxic mediators and distinct subpopulations producing IFN- γ and IL-17. Dimova and colleagues revealed that the immune response after bacillus Calmette–Guérin vaccination of infants can drive $\gamma\delta$ T cells into an effector cell type that produce IFN- γ and mediate cytotoxicity. Costa and colleagues found that the extracellular malaria merozoite is a new activator and target of the antiparasitic activity of $\gamma\delta$ T cells. With granulysin rather than perforin, V γ 9V δ 2 T cells can inhibit multiplication of *Plasmodium falciparum* blood stages *in vitro*. By establishing an animal model, Wang and colleagues explored that recall expansion of V γ 2V δ 2 T cells in *Mycobacterium*- or *Listeria*-infected monkeys depends on Th17-associated cytokines from host together with primed and boosted microbial loads.

Ag recognition is important in $\gamma\delta$ T cell-mediated virus and bacteria clearance. Li and colleagues demonstrated that $\gamma\delta$ T cells are effector cells in parasite elimination in AIM (apoptosis inhibitor expressed by macrophages)^{-/-} mice. Legrand and colleagues observed that human eosinophil granulocytes express a functional $\gamma\delta$ TCR complex and may contribute to defense to *Mycobacteria* infection and tumors.

Rajoriya and colleagues reported that the frequency of CD161⁺ $\gamma\delta$ cells in peripheral blood is reduced in HCV-infected patients. Furthermore, CD161⁺ $\gamma\delta$ cells from HCV⁺ patients expressed higher levels of CXCR6, CD38 and CD69 compared to those of healthy populations. It revealed that CD161⁺ $\gamma\delta$ cells are more active and with higher 'liver homing' potential concentrated in the liver in HCV patients. Wu and colleagues detected that the frequency of V δ 2 $\gamma\delta$ T cells is significantly decreased in immune-active chronic hepatitis B patients both in the blood and in the liver. Moreover, $\gamma\delta$ T cells are more active and secrete more TNF- α in immune-active chronic hepatitis B patients when compared with healthy control patients. V δ 2 $\gamma\delta$ T cells are closely linked with liver damage and may play a protective role. Pauza and colleagues observed that lymphocytes were severely impacted in HIV controllers (i.e., patients whose HIV infection does not progress to AIDS disease), although a unique V γ 2V δ 2 T-cell population that sustains the phosphoantigen response can guard against damage by immunodeficiency viruses.

FUNCTION IN ANTITUMOR REACTION

$\gamma\delta$ T cells are potent effectors of innate immunity and are involved in antitumor immune responses.^{18,19,55–57} A lot of studies are currently focusing on the antitumor effector subset of human $\gamma\delta$ T cells. Knight and colleagues examined the potential of $\gamma\delta$ T cells to lyse tumor targets *in vitro* and reported that V δ 1 $\gamma\delta$ T cells showed specific cytotoxicity to many tumor cell lines, the most prominent of which were glioblastoma, myeloma, colon and lymphoma. Similarly, Devaud and colleagues reported that V δ 1 $\gamma\delta$ T cells significantly suppress colon cancer cells. There are also reports focusing on the V δ 2 $\gamma\delta$ T cell-mediated tumor immunity. Gertner-Dardenne and colleagues reported mice receiving V γ 9V δ 2 T cells exhibited superior survival rates compared to untreated controls upon transfer of acute monocytic leukemia cells. The CD226 ligands poliovirus receptor and Nectin-2 play an important role as tumor cell-expressed targets for the V γ 9V δ 2 T cell-mediated killing of acute monocytic leukemia blasts.

Molecular mechanisms underlying lymphoma recognition by $\gamma\delta$ T cells remain unclear. Lança and colleagues reported that the expression levels of UL16-binding protein 1 (ULBP1) determine lymphoma

susceptibility to $\gamma\delta$ T cell-mediated cytotoxicity. Consistently, a blockade of NKG2D, the receptor for ULBP1 expressed on all V γ 9 $\gamma\delta$ T cells, significantly inhibits lymphoma cell killing. Specific loss-of-function studies demonstrate that the role of ULBP1 is non-redundant, highlighting a thus far unique physiological relevance for tumor recognition by $\gamma\delta$ T cells. Importantly, they observed a very wide spectrum of ULBP1 expression level in primary biopsies obtained from lymphoma and leukemia patients. This study also proposes that ULBP1 may be used as a leukemia/lymphoma biomarker in upcoming clinical trials.

FUNCTION IN TISSUE REPAIR AND INFLAMMATION

Skin $\gamma\delta$ T cells were found to be critical in wound healing,^{58–62} and IL-17 was reported to be elevated in patients with inflammatory skin diseases.⁶³ However, the role of IL-17 in cutaneous wound healing, and whether it is produced by skin $\gamma\delta$ T cells, are less clear. Here, Büchau and colleagues reported that cultured skin $\gamma\delta$ T cells rapidly produce IL-17A upon TCR activation. Furthermore, IL-23 and IL-1 β exposure led to IL-17A secretion. Using an *in vivo* wounding model, they found substantial and transient IL-17 production by skin $\gamma\delta$ T cells, with an IL-17 deficiency leading to a defect in wound repair. These results implied a critical role of $\gamma\delta$ T cell-produced IL-17 in cutaneous immunity and wound healing.

One example of $\gamma\delta$ T-cell dysfunction is the skin complications associated with obesity and metabolic disease. Taylor and colleagues reported that elevated glucose impairs the ability of skin $\gamma\delta$ T cells to proliferate, and leads to a loss of $\gamma\delta$ T-cell responsiveness to stimulation.⁶⁴ This suggests that the environment in metabolic disease has negative effects on the homeostasis and functionality of skin lymphocytes and renders them unresponsive to epithelial damage, and may provide new and previously unrecognized therapeutic targets for treating complications associated with metabolic disease.

IMMUNOTHERAPY

Some preclinical investigations indicated that $\gamma\delta$ T cells may play a certain role in antitumor effects and many phosphoantigens can promote the function of $\gamma\delta$ T cells *in vitro*.^{57,65} Capietto and colleagues reported that bromohydrin pyrophosphate-treated V γ 9V δ 2 T cells can bypass TGF- β -mediated immunosuppression in current cancer immunotherapy. Castella and colleagues observed that dysfunction of V γ 9V δ 2 T cells in multiple myeloma patients can be fully recovered by zoledronic acid and tumor-associated antigen-treated DCs. Furthermore, Cimini and colleagues identified that zoledronic acid can enhance the antitumor response to glioma cell lines of V δ 2 T lymphocytes.

Animal models have been established to identify the functions of $\gamma\delta$ T cells in tumor therapy. Siegers and colleagues developed a preclinical xenograft model of chronic myeloid leukemia to investigate the cytotoxicity of $\gamma\delta$ T cells. With this model, they also found that the cytotoxicities of V δ 1 and V δ 2 T cells are different when compared against Philadelphia chromosome-positive and B-chronic lymphocytic leukemia-derived leukemic cells. Laws and colleagues used a marmoset model to assess the antitumor function of IPH1201 (a phosphoantigen, can specifically activate $\gamma\delta$ T cells) *in vivo*. Subcutaneous dosing with IPH1201 plus IL-2 caused specific expansion of V γ 9 T cells, suggesting marmoset may be an appropriate model for testing IPH1201 as an immunotherapy.

As accumulating evidence shows that $\gamma\delta$ T cells, especially V γ 9V δ 2 T cells, have antitumor effects both *in vitro* and in animal models, researchers have tested their function in clinical therapies in

patients.^{66–68} The frequency of $\gamma\delta$ T cells is correlated to the patients' clinical survival rate. Tanaka and colleagues reported that in advanced renal cell carcinoma, a higher survival rate after surgery is correlated to higher $\gamma\delta$ T-cell frequency in patients' peripheral blood.

Phosphoantigen and fungal immunomodulators play an important role in $\gamma\delta$ T cell-mediated immunotherapy. Dunne and colleagues revealed that HMBPP-activated V γ 9V δ 2 T cells strongly promotes Th1 responses. Kunzmann and colleagues observed that zoledronic acid plus IL-2-treated $\gamma\delta$ T cells can induce significant immunological responses, but the objective clinical response rate is low and restricted to hematological malignancies. In order to improve antitumor function, Binda and colleagues optimized the immunotherapeutic potential of zoledronate-activated V γ 9V δ 2 T cells in many ways. Li and colleagues observed that rapamycin modulated IPP/IL-2-induced V γ 2V δ 2 T-cell proliferation in a dose-dependent manner.

$\gamma\delta$ TCRs are quite important in the recognition of tumor cells. Marcu-Malina and colleagues retrovirally transduced tumor-specific high-affinity $\gamma\delta$ TCRs into human $\alpha\beta$ T cells. After stimulation by phosphoantigens, this kind of modified $\alpha\beta$ T cells can be effective against a broad panel of cancer cells while ignoring normal cells. Pérez and colleagues established a prognostic value system to test $\gamma\delta$ T-cell responses in breast cancer patients during zoledronate therapy.

In addition to tumor immunotherapy, $\gamma\delta$ T cells are also considered in other diseases. Hawchar and colleagues reported that TEMRA (terminally differentiated effector memory, CD45RA⁺CD27⁻) V δ 2⁻ $\gamma\delta$ T lymphocytes are endowed with anticytomegalovirus potential in kidney transplant recipients and can be expanded by IL-2 and IL-15. Dorp and colleagues detected that $\gamma\delta$ T cells present in patients after allogeneic transplantation have the potential to eradicate cytomegalovirus-infected fibroblasts and to spread an immune response to $\alpha\beta$ T cells *via* DC maturation. Bol-Schoenmakers and colleagues found that murine intestinal $\gamma\delta$ T cells play a regulatory role during establishment of allergic sensitization to peanuts, suggesting that targeting intestinal $\gamma\delta$ T cells may provide preventing and therapeutic strategies for food allergy.

SUMMARY REMARKS

Where are we heading to from here with respect to the biological function of $\gamma\delta$ T cells? After several open discussion sessions, it became evident that one important area is to bring together the results from animal studies with those gained from human studies. Otherwise, two different languages ('mice' and 'human') would hurdle the understanding of biological function of $\gamma\delta$ T cells. Defining the ligands of $\gamma\delta$ T cells is still far from the reach. Cytokine production by $\gamma\delta$ T cells may regulate the function of $\alpha\beta$ T cells and other antigen-presenting cells, and this area may open the window for understanding the whole picture of immune responses. The role of $\gamma\delta$ T cells in emerging and chronic infections needs more attention and potential therapy may derive from manipulating the function of these $\gamma\delta$ T cells. Finally, the potential therapeutic role of $\gamma\delta$ T cells in malignancy has clearly shed new light in tumor immunotherapy. No doubt, exciting results will be further discussed at the next 2012 $\gamma\delta$ T cell Conference to be held in Rome, Italy. All roads lead to Rome, and the same is true for the mysterious $\gamma\delta$ T cells.

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