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REGULATION OF SKIN CELL HOMEOSTASIS BY GAMMA DELTA T CELLS

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Gamma Delta T Cells
3.1. DETC antigen recognition
3.2. Gamma delta T cell signaling
3.2.1. TCR/CD3 complex
3.2.2. Protein tyrosine kinases
3.2.3. Downstream linker and effector pathways
3.3. Cytokines and chemokines
3.3.1. Cytokines produced by gamma delta T cells
3.3.2. Cytokines produced by keratinocytes and Langerhans cells
3.3.3. Chemokines
4. Gamma Delta IEL and Tissue Homeostasis
4.1. DETC play a role in keratinocyte proliferation during wound repair
4.1.1. DETC may also play a role in inflammation associated with epidermal repair
4.2. Intestinal gamma delta IEL play roles in maintaining the intestinal mucosa
4.3. Lung IEL are involved in airway homeostasis
5. Gamma Delta T Cell Functions in Humans
6. Summary and Perspective
7. Acknowledgements
8. References

1. ABSTRACT

Although innate T lymphocytes such as gamma delta T cells have been extensively studied, their biological role has remained an enigma to researchers for many years. However, recent advances have begun to explain their complex role in the immune system. Gamma delta T cells are often the major T cell population in epithelial tissues such as the skin, gut, and lung where they have been implicated in maintaining tissue integrity, defending against pathogens, and regulating inflammation. The gamma delta T cells that reside in the skin are a prototypical intra-epithelial lymphocyte (IEL) population. These skin gamma delta T cell receptor (TCR)-expressing cells are named dendritic epidermal T cells (DETC) for their unique dendritic morphology. Using their gamma delta TCR, DETC recognize an unknown ligand expressed by stressed or damaged keratinocytes. Activated DETC exhibit effector functions such as cytokine production, cytolysis, and proliferation *in vitro*. Recent findings have shown that upon activation by damaged keratinocytes, DETC produce a key keratinocyte growth factor for wound repair called fibroblast growth factor 7 (FGF-7). FGF-7 is produced *in vitro* and *in vivo*, suggesting that DETC might play an important role in the biological function of wound repair. Indeed a delay in wound closure and a decrease in the proliferation of keratinocytes at the wound site have been observed in mice lacking gamma delta T cells. In addition to effector functions attributed to DETC, it has also been suggested that gamma delta T cells such as DETC have regulatory roles such as initiating or inhibiting inflammation. This is supported by the findings that DETC produce chemokines and cytokines. Control of the inflammatory response in the epithelium may provide another mechanism to reestablish homeostasis after a biological insult such as wound infliction. Understanding the function of DETC may be useful in the development of future therapies for chronic wounds and the maintenance of skin homeostasis.

2. INTRODUCTION

Although alpha beta T lymphocytes make up the majority of T cells in murine lymphoid compartments, gamma delta T cells are more abundant in epithelial tissues such as the skin, lung, and reproductive tract (1). Gamma delta T cells, along with NK T cells, encompass a unique group of cells collectively called innate T lymphocytes. Gamma delta T cells have T cell receptors (TCRs) with a unique usage that correlates with their location (reviewed in (2-5)). Although the various roles they play may differ according to their location, roles for gamma delta T cells in tissue homeostasis have recently been identified.

During ontogeny, thymocytes expressing the gamma delta TCR are the first to develop. Distinct populations of murine gamma delta T cells migrate to various organs during thymic development in a series of waves (6). Each wave of T cells has a unique TCR with limited diversity. The first T cells that arise during day 14 of embryonic development are Vgamma3 Vdelta1 TCR-expressing cells that migrate to the skin (reviewed in (7)). Next, the Vgamma4 Vdelta1 TCR-expressing T cells are

seeded in the reproductive tract and tongue (8). A more diverse T cell population expressing Vgamma1 or Vgamma2 colonizes the lymphoid system and the lung (5, 9, 10). Extrathymically derived Vgamma5 cells predominate in gut cryptopatches (reviewed in (11)). In humans there is a population of gamma delta T cells with limited diversity in the peripheral blood expressing a Vgamma9 Vdelta2 TCR. These gamma delta T cells recognize nonpeptidic phosphoantigens such as pyrophosphates (reviewed in (12)). This Vgamma9 Vdelta2 subset expands extrathymically. The expansion has been suggested to be antigen driven instead of being due to positive selection since this population only develops in the fetal thymus (13).

The Vgamma3 Vdelta1 T cells that migrate to the murine skin are a prototypical gamma delta intra-epithelial lymphocyte (IEL) population. Named dendritic epidermal T cells (DETC) after their dendritic morphology and residence in the epidermal compartment of the skin, DETC have close interactions with keratinocytes. DETC recognize transformed, stressed, or damaged keratinocytes, by means of their TCR, thus they can respond to different biological insults giving them great heterogeneity of response (14-16) despite their lack of diversity. The canonical Vgamma3 Vdelta1 TCR is not expressed by cells in any other tissue, suggesting that the antigen specificity of the TCR is specific to skin. Studies using Vgamma3 deficient mice have shown that DETC expressing a Vgamma1.1Vdelta1 TCR still migrate to the skin (17). This replacement TCR is similar in conformation to the original DETC TCR and responds to stressed keratinocytes (17). An extensive effort has been underway to identify the DETC ligand in the hopes of better understanding the activation requirements and biological functions of DETC.

Innate T lymphocytes, such as DETC, respond rapidly to antigenic stimulation. This may provide a primary defense in which gamma delta IELs recognize self-antigens expressed by damaged, stressed, or transformed resident tissue cells. Both effector and regulatory roles have been described for gamma delta IELs. Effector responses such as growth factor and cytokine production may have direct effects on biological insults such as damage to the epithelium, tumor growth, or infection. Recent advances have characterized certain gamma delta T cell populations with regulatory roles, which may indirectly adjust the cytokine milieu by selecting the cells that can enter the micro-environment. Control of the inflammatory response in the epithelium may provide another mechanism to reestablish homeostasis after a biological insult. This review will focus on some of the key aspects of gamma delta T cell function in the epithelium, centering on gamma delta T cell activation, signaling, and production of cytokines, growth factors, and chemokines. Recent advances in the effector and regulatory roles that gamma delta T cells play in the skin, gut, and lung will be addressed along with the importance of these studies to human disease and future therapies.

3. GAMMA DELTA T CELLS

Gamma delta T cells do not recognize antigen in the context of classical major histocompatibility complex (MHC) presentation, but become activated in a manner distinct from alpha beta T cells (reviewed in (4, 18, 19)). The activation of gamma delta T cells originates with the TCR complex which, along with accessory molecules, provides the signals for effector functions such as growth factor production and regulatory functions such as chemokine production. Some of these DETC-produced or keratinocyte-produced factors are also necessary for the survival of the gamma delta T cell within the epidermal micro-environment.

3.1. DETC antigen recognition

Antigen recognition by gamma delta T cells has been investigated, however not much progress has been made in determining the specificity of the skin gamma delta TCR. The Thy1-positive T cell population in the murine skin expresses a canonical gamma delta TCR (20-22). While most T cells have extensive junctional diversity due to N nucleotide insertions or exonucleolytic action, DETC have identical TCR junctional regions. Expression of the Vgamma3 Vdelta1 DETC TCR is rarely observed in thymocytes after birth (23). To show that DETC originate in the thymus, young athymic nude/nude mice were shown to have no Vgamma3 Vdelta1-expressing DETC in the epidermis. When day 16-17 fetal thymocytes, purified by antibodies specific for the TCR or CD3, were transferred into young nude/nude mice, DETC with the canonical TCR accumulated in the skin (24-26). Further evidence of the thymic origin of Vgamma3 DETC was observed when fetal liver hematopoietic stem cells were used to repopulate fetal thymic lobes with Vgamma3-expressing cells (27).

The restricted expression of the Vgamma3 Vdelta1 TCR to T cells in the skin suggests that DETC recognize an antigen restricted to the epidermis. The expression of a canonical TCR also suggests that the ligand may be a single antigen. Recognition of this antigen seems to be crucial since T cells with a similar TCR conformation still develop in mice lacking the Vgamma3 TCR chain (17). It is also likely that DETC recognize a self antigen since the activation or development of DETC does not require classical processing or presentation by MHC, unlike alpha beta T cells (28). Since DETC recognize self antigens on distressed epithelial cells, they may be a unique regulatory population that can monitor cells to maintain homeostasis in the epidermal compartment.

To identify which cells in the epidermis express the antigen that activated DETC, co-culture experiments were performed. Chemically or physically stressed keratinocytes, but not other cell types, activate DETC (14, 15). This stimulation can be blocked by antibodies specific for the Vgamma3 TCR, thus activation of DETC by keratinocytes is dependent on TCR ligation. The ligand is cell-associated since keratinocytes plated in the upper well of a trans-well can not activate the DETC in the lower compartment (14). Although cell-cell contact enhances DETC activation *in vitro*, classical co-stimulatory molecules used by alpha beta T cells, such as CD28, are not required (reviewed in (3, 19)).

Since some gamma delta T cells recognize antigen directly, not requiring accessory cells (19), the

recognition of antigen by gamma delta T cells may be similar to the recognition of antigen by antibody. In support of this, the gamma delta TCR CDR3 length is more like the CDR3 length of immunoglobulin molecules than alpha beta TCRs (29). However, the structure of the CDR3 from the delta chain is similar in conformation to the variable region of the alpha chain (30). Although, some effort has gone into trying to define the keratinocyte antigen that activates DETC, the identity of the activating self-antigen is still unknown. We have characterized a low-molecular weight fraction from stressed keratinocytes that stimulates DETC (our unpublished results). This product does not activate gamma delta cells from other tissues or alpha beta cells. However, further work is needed to identify the antigen.

3.2. Gamma delta T cell signaling

3.2.1. TCR/CD3 complex

The basic structure of the alpha beta and gamma delta TCR are similar in that they both contain two TCR chains surrounded by a CD3 complex. The CD3 complex contains Immuno-Tyrosine Activation Motifs (ITAMs) which allow for tyrosine phosphorylation and provide docking sites for kinase and linker proteins to form the base of a signaling cascade. In alpha beta T cells, the alpha and beta TCR chains are flanked by CD3 gamma epsilon and delta epsilon dimers, with each chain providing one ITAM and also a CD3 zeta homodimer with each chain providing three ITAMs. The members of the TCR-associated signaling complex differ slightly between the two subsets of T lymphocytes. Gene deletion experiments have shown that both alpha beta and gamma delta TCR require CD3 epsilon,gamma, and zeta for development or maturation (reviewed in (31)). However, CD3 delta deficient mice lack alpha beta T cells but have normal numbers of gamma delta T cells suggesting that the gamma delta TCR does not require CD3 delta for function (32, 33). Analysis of gamma delta TCR transgenic cells and gamma delta intestinal IELs showed an absence of CD3 delta in the TCR complex (34). The main ITAM-containing component of the TCR signaling complex is CD3 zeta. In alpha beta T cells CD3 zeta forms a homodimer in the signaling complex of both the pre and mature alpha beta TCR providing 6 ITAM motifs. Gamma delta T cells require CD3 zeta as evidenced by a paucity of gamma delta T cells in CD3 zeta deficient mice, however in gamma delta T cells, CD3 zeta is able to homodimerize or heterodimerize with Fc epsilon Rgamma1 (35). Heterodimerization with Fc epsilon Rgamma1 reduces the number of available ITAMs from six to four as Fc epsilon Rgamma1 provides only one ITAM. The requirement of Fc epsilon Rgamma1 is not absolute as deletion of Fc epsilon Rgamma1does not prevent gamma delta development (36). Recently it has been suggested that presence of Fc epsilon Rgamma1 in the gamma delta TCR complex may indicate activated cells as it was found in *in vitro* stimulated but not *ex vivo* lymph node gamma delta T cells (34). *Ex vivo* gamma delta+ intestinal IEL do contain Fc epsilon Rgamma1 in the TCR complex (35, 37). This has been confirmed by recent gene expression analysis of gamma delta iIEL (38). This is consistent with the hypothesis that tissue resident gamma delta cells may have constitutive low level activation due to the continuous local presence of antigen.

3.2.2. Protein tyrosine kinases

The signaling pathways engaged by alpha beta T cells upon TCR recognition of MHC loaded with peptide antigen are well established. At the surface, the alpha beta TCR and the associated co-receptors CD4 or CD8 engage MHC with peptide. The co-receptors bring in the src-family protein tyrosine kinase (PTK) Lck which phosphorylates ITAM motifs in components of the TCR-associated CD3 complex. Lck activity is controlled in part by CD45 phosphatase which dephosphorylates the c-terminal autoinhibitory tyrosine of Lck. Antigen recognition is less well defined for gamma delta T cells. DETC do not require antigen presentation by MHC (14, 15). Although some gamma delta T cells do express coreceptors (largely CD8), DETC do not. Lck and CD45 are, however, important for DETC expansion and function as mice deficient for either protein have a ten fold reduction in numbers of DETC (39). The Src family tyrosine kinase Fyn is able to partially rescue alpha beta T cell development in Lck deficient mice, but is not required for DETC development as Lck Fyn doubly deficient mice show no further reduction in numbers of DETC as compared with mice deficient for Lck alone (40). Confocal microscopy of a transfected pro-T cell line suggests that Lck may not colocalize with the gamma delta TCR during development (41). Taken together, this suggests that the Src-family kinases may be important for expansion but not necessarily the development of DETC. The Syk/ZAP-70 family of PTK directly binds to ITAMs of the CD3 complex and serve both as linker proteins and kinases and are activated subsequently to Lck/Fyn. DETC show variable dependence upon the Syk/ZAP-70 family as ablation of either gene results in a significant reduction in DETC, with those remaining having abnormal morphology (42, 43). Rag deficient mice reconstituted with Syk deficient T cells fail to develop DETC or gamma delta intestinal IEL but are able to develop splenic gamma delta T cells (43). Because Syk is utilized by Fc epsilon Rgamma1, this suggests a unique and central role for Syk in tissue resident gamma delta T cells and underscores that studies using peripheral gamma delta cells may not represent epithelial gamma delta T cells.

3.2.3. Downstream linker and effector pathways

The CD3 complex serves as the base of several signaling cascades leading to calcium mobilization, the activation of MAP kinases, and transcriptional activity. The linker protein LAT provides docking sites for the activation of several cascades. Mice deficient in LAT do not develop alpha beta T cells (44). While there are some gamma delta thymocytes in LAT-/- mice, DETC fail to develop (44). Further genetic dissection of the LAT phosphorylated tyrosine residues suggests that while Y6F is required for alpha beta but not gamma delta development, mutation of Y7/8/9F prevents development of circulating gamma delta cells (45). The guanine nucleotide exchange factor Vav1 is also required for the development of alpha beta but not gamma delta T cells. Although Vav1 is not required for gamma delta development, it plays a critical role in gamma

delta intestinal IEL activation as Vav1 deficient cells are unable to be activated in response to TCR stimulation (46).

Comparisons of effector signaling pathways in alpha beta and gamma delta cells have been recently undertaken. Hayes and Love (34) showed that circulating transgenic gamma delta T cells flux calcium with faster kinetics and higher amplitude at lower levels of TCR stimulation when compared to alpha beta T cells. Similarly the MAP kinases ERK and JNK also had greater intensity and duration of phosphorylation in gamma delta T cells. Studies of human circulating Vgamma9Vdelta2 T cells showed similar increases in ERK phosphorylation duration and intensity in response to IL-2 stimulation (47) and showed increased STAT4 DNA binding and IFN-gamma production in gamma delta cells, pathways that are not normally activated in alpha beta cells upon IL-2 stimulation. This suggests that gamma delta T cells may have unique responses to activation. It is also important to note that the signaling studies completed to date have used circulating gamma delta T cells that may not be representative of the events that occur in tissue resident gamma delta T cells, the major population of gamma delta T cells in the body.

3.3. Cytokines and chemokines

The unique antigen recognition by gamma delta T cells and their unique TCR structure suggest that the functional outcome of the activation of DETC, and gamma delta T cells in general, may also be distinctive. One read-out of this activation is the production of soluble factors such as cytokines, chemokines, and growth factors. It is becoming increasingly clear that survival and maintenance of cells and tissues is not an intrinsic property of the cells themselves, but requires a number of different exogenous factors. A crucial role for cytokines in homeostasis has already been clearly demonstrated for a number of cell types in secondary lymphoid tissues, including alpha beta T cells (48) and NK cells (49). Such a role for cytokines, chemokines and growth factors is also evident for gamma delta T cells in the skin.

3.3.1. Cytokines produced by gamma delta T cells

DETC clones were first shown to produce IL-2 and IL-3 in response to both mitogenic stimulation and anti-TCR antibodies (50). DETC lines also have a requirement for IL-2 for their growth and survival (51), suggesting an autocrine function for this cytokine. Additionally, activated DETC have been shown to produce a number of other cytokines. In particular, GM-CSF, IFN-gamma and TNF-alpha are highly upregulated, at least at the mRNA level (52), following activation. DETC therefore produce an array of proinflammatory cytokines of the Th1 type in response to TCR-mediated activation. Interestingly, GM-CSF has been shown to be mitogenic for keratinocytes and promotes survival and maturation of Langerhans cells. As such this cytokine is likely to be involved in keratinocyte repair. IFN-gamma is also known to affect Langerhans cells by modulating their adhesion molecule expression and function (53, 54). Other cytokines such as TGF-beta1, are expressed constitutively (52), presumably for cell maintenance in normal, undamaged skin. In contrast to GM-CSF, TGF-beta inhibits proliferation of many cell types, including keratinocytes, but at the same time is able to stimulate migration of keratinocytes, induces expression of integrins necessary for this keratinocyte migration (55) and is crucial for Langerhans cell development (56). Proliferation and migration of keratinocytes and Langerhans cells is crucial to maintenance, or restoration, of skin homeostasis. A role for cytokines in this process is thus evident.

3.3.2. Cytokines produced by keratinocytes and Langerhans cells

Keratinocytes and Langerhans cells are in close contact with DETC in the skin and it stands to reason that if cytokines produced by DETC affect growth and survival of keratinocytes and Langerhans cells, production of cytokines by these cells could in turn have an effect on DETC. This is indeed the case. Keratinocytes produce IL-7, a cytokine that has been shown in numerous studies to be crucial for survival of DETC (57, 58) and to induce proliferation of DETC (59, 60). TNF-alpha is also produced by keratinocytes (61), a cytokine that has been shown to enhance the IL-7 response of DETC (62). The interplay between DETC and keratinocytes is further highlighted by the IFN-gamma induced upregulation of IL-7 secretion by keratinocytes (63); IFN-gamma being produced by activated DETC (as mentioned above). IL-15 is another cytokine that facilitates growth of DETC (64) and is also produced by keratinocytes (65). In fact keratinocytes produce a variety of cytokines (reviewed in (66)) as do the third major epidermal population, the Langerhans cells. These locally produced cytokines are crucial to the communication between DETC, keratinocytes and Langerhans cells and, as such, play a pivotal role in maintaining epidermal homeostasis.

3.3.3. Chemokines

DETC also produce a number of chemokines and growth factors. The keratinocyte growth factors play an important role in skin maintenance. These will be dealt with in detail in the following section so will not be discussed further here. Chemokines are important factors in maintenance of the integrity of the skin. DETC produce several chemokines in response to activation. Using quantitative RNase protection assay, lymphotactin, MIP-1alpha, MIP-1-beta and RANTES were all induced *in vitro* in activated DETC clones (67). Lymphotactin was found to be the most abundant of the evaluated chemokines. On the other hand, MCP-1 could not be detected (67). These results have been confirmed and extended in recent *in vivo* studies (our unpublished data). Others have found high levels of MCP-1 early following skin wounding, peaking at 24 hours and declining thereafter. However the MCP-1 appears to be keratinocyte derived. This, together with the presence of chemokine receptors on resident cells in the skin, including keratinocytes, indicates that chemokines contribute to the maintenance of T cell-keratinocyte interactions in both normal and damaged skin.

Maintenance of keratinocyte, Langerhans and DETC populations is likely controlled, at least in part, by local production of these cytokines, chemokines and

growth factors, under both normal conditions, as well as following stress or damage to skin epithelia.

4. GAMMA DELTA IEL AND TISSUE HOMEOSTASIS

Tissue repair is a complex process that involves the precise timing of a variety of factors and cellular interactions. Delays in the progression of wound healing can lead to chronic nonhealing lesions, such as those commonly found in diabetic patients (68). Upon activation, gamma delta T cells exhibit effector functions such as growth factor and cytokine production *in vitro* suggesting that gamma delta IEL are involved in tissue homeostasis and repair (18). Recent advances have further characterized the effector and regulatory roles that gamma delta T cells play in the maintenance of epithelial integrity *in vivo*.

4.1. DETC play a role in keratinocyte proliferation during wound repair

Wound repair involves the careful coordination of cells, factors, and extracellular matrix to reestablish the main barrier providing protection for the body (reviewed in (68)). Epidermal and dermal regeneration occurs concurrently in steps involving inflammation, proliferation, and matrix remodeling. Keratinocytes undergo proliferation and migration during the initial stages of wound repair continuing until the dermal surface is covered and a new basement membrane has formed. During inflammation, platelets along with other cells are involved in the recruitment of neutrophils and monocytes via chemotactic factors. Monocytes differentiate into macrophages and proliferate to help form granulation tissue. Lastly matrix is formed and granulation tissue is remodeled into scar tissue.

Keratinocytes, which make up over 90% of the cells in the epidermis, have close interactions with DETC. In epidermal sections one DETC can be observed using long dendrites to make interactions with a number of keratinocytes (our unpublished data). DETC produce cytokines and proliferate in response to contact with damaged or stressed keratinocytes *in vitro* (14, 15). This indicates that there may be a functional interaction between these epidermal cell types *in vivo*. It is suggested that upon damage to the epidermis, keratinocytes express a self-antigen that is recognized by DETC via their gamma delta TCR. These activated DETC produce cytokines and factors that may be involved in keratinocyte proliferation during wound repair. This allows keratinocytes to proliferate in order to regenerate the epidermal cover.

To examine the role of DETC on keratinocyte proliferation within a biological system, a mouse model of wound repair was established. Recent work has focused on characterizing the role that DETC play within this model system. DETC adjacent to the injured keratinocytes lose their characteristic dendritic shape within 24 hours post-wounding (69). These gamma delta T cells upregulate RNA expression for cytokines and growth factors, and increase cell surface expression of early activation markers such as CD25 and CD69 (69, 70). Taken together, these results indicate that DETC isolated from a site of epithelial damage exhibit effector functions. These effector roles played by DETC during wound repair may be important for wound closure. Therefore, mice lacking gamma delta T cells (TCR delta knock-out mice) have been examined for defects in wound repair. TCR delta knock-out mice have a 2 to 3 day delay in wound closure as compared to wild-type mice (69). Histological analysis of the wounds from TCR delta knock-out mice show decreased hyperthickening of the epidermis and lower levels of keratinocyte proliferation than wounds from wild-type mice (69). Similar results have been observed in OT-1 Rag knock-out mice, which only have T cells expressing the Valpha2 Vbeta5 TCR, further emphasizing the importance of wild-type DETC in wound repair (69).

A likely candidate for the induction of keratinocyte proliferation is keratinocyte growth factor-1 (FGF-7), which binds the high affinity keratinocyte growth factor receptor (FGFR2-IIIb) (71, 72). FGFR2-IIIb is only expressed on epithelial cells, however FGF-7 is not produced by keratinocytes, and thus must be obtained from a nearby source (73). Mice containing the dominant negative FGFR2-IIIb have delays in wound repair further implicating FGF-7 in wound healing (74). Interestingly, the FGF-7 knock-out mice do not have a delay in wound closure (75). This has led to the discovery of another factor that binds FGFR2-IIIb designated keratinocyte growth factor 2 (FGF-10) (76). The FGF-10 knock-out mice are embryonically lethal due to a defect in lung development, therefore it is not known whether a FGF-7/FGF-10 double knock-out would display the same wound healing defects as those containing the dominant negative FGFR2-IIIb. However, exogenous application of either FGF-7 or FGF-10 accelerates wound repair suggesting that either is sufficient for wound closure (77-80).

Upon TCR stimulation *in vitro*, DETC produce FGF-7 (81). Growth factors that bind the FGFR2-IIIb appear to be the major factors produced by activated DETC that support keratinocyte proliferation since a FGFR2-IIIb neutralizing peptide blocks DETC-induced keratinocyte growth (81). Recently both FGF-7 and FGF-10 expression were detected in DETC isolated from wild-type wound sites (69). To examine whether FGF production by DETC is the mechanism for accelerating wound repair, a skin organ culture system was established. Keratinocytes proliferated at the wound site of wild-type skin, but not skin from TCR delta knock-out mice (69). However the diminished keratinocyte proliferation in TCR delta knock-out skin could be restored to wild-type levels by adding recombinant FGF-7 or activated DETC to the well (69). These findings indicate that DETC play a key role in the early keratinocyte proliferation that occurs during wound repair via the production of keratinocyte growth factors.

4.1.1. DETC may also play a role in regulating inflammation associated with epidermal repair

As mentioned earlier, upon activation *in vitro*, DETC can produce chemokines such as MIP-1 alpha, MIP-1 beta, MCP-1, RANTES, and lymphotactin (67). Furthermore, DETC-produced lymphotactin attracts alpha beta T cells *in vitro* (67). Thus gamma delta T cells have been implicated in the regulation of other cell populations

such as neutrophils and alpha beta T cells during inflammation. During wound repair, the inflammatory response is essential to rapid and complete wound healing. Dysregulation of inflammatory cell entry into the wound site would cause a delay in the progression of wound closure or could potentially cause a wound to remain chronically open. Thus DETC potentially play a crucial role in the restoration of skin homeostasis via chemokine production. Other gamma delta populations have been implicated in regulatory roles as well. For example, the gamma delta T cells that reside in the intestine seem to be an "activated yet resting" population of cells (82). Gene usage examined by serial analysis of gene expression (SAGE) has identified RANTES as one of several chemokines that is highly expressed by IELs of the intestine (82). Other chemokines with high expression levels include MIP-1 alpha and MIP-1 beta (82). Other cases in which inflammation is important to regaining homeostasis is during infection. During *Listeria monocytogenes* infection, mice that lack gamma delta cells had reduced levels of MCP-1, which led to the slower replacement of neutrophils by macrophages and increased levels of tissue necrosis (83).

Gamma delta T cells have been reported to down-regulate inflammation as well. Regulatory cell populations resolve inflammation after the infectious agent has been eradicated. If the adaptive immune response proceeds unchecked, allergy or chronic inflammation such as cutaneous inflammatory diseases may develop. It was recently discovered that NOD or FVB mice that lack gamma delta T cells have increased evidence of spontaneous dermatitis (84). The disease is presented with large numbers of alpha beta T cells in the skin (84). Allergic and irritant contact dermatitis are also increased in these strains of TCR delta knock-out mice (84). In other reports mice lacking gamma delta T cells exhibited increased contact hypersensitivity responses (85). This increase in contact hypersensitivity indicates that again gamma delta T cells are down-regulating CD8+ T cells that elicit the responses (85). The regulatory roles of DETC are complex and may depend on both the type of biological assault and the timing of DETC involvement.

4.1.2. DETC regulate the development of skin cancer

It has long been observed that DETC have cytolytic function. DETC can recognize and lyse malignant epithelial cells *in vitro* (86, 87), which suggests they may be functional effectors against malignant skin tumors. Mice either intradermally inoculated with carcinoma cells or topically treated with skin cancer-inducing chemicals exhibited increases in skin cancer when gamma delta T cells were absent (88). Studies into the mechanism of malignant cell lysis found that recognition *in vitro* involved the interaction of rae-1 expressed on carcinoma cells and NKG2D expressed on DETC (88). Rae-1 is a self-molecule expressed by both skin exposed to carcinogen-inducing chemicals and by tumor cell lines but not by undamaged epithelial cells (88). NKG2D is a nonclassical MHC molecule used by NK and T cells. The NKG2D/rae-1 interaction represents an example of a stress molecule expressed by epithelial cells for gamma delta T cell recognition. The NKG2D ligands, MICA and MICB have already been well-characterized in humans. Stress increases the expression of these molecules on human epithelial cells and subsequent Vdelta1 T cell recognition (89). Molecules such as MICA in humans and rae-1 in mice may be indicators used by normal cells undergoing stress to signal gamma delta T cells of potential danger within the tissue.

4.2. Gamma delta IEL play roles in maintaining the intestinal mucosa

Several types of IEL reside in the small intestine including both alpha beta and gamma delta T cells. The role of the gamma delta T cells in the intestine is controversial (90-96). Like activated DETC, activated intestinal gamma delta T cells can produce FGF-7, while intestinal alpha beta IEL can not (14, 81, 97). Studies done in a dextran sodium sulfate (DSS)-induced model of murine colitis have shown that large numbers of gamma delta T cells are detected at the sites of DSS-induced damage (97). Like DETC in the skin, intestinal gamma delta IEL increase RNA expression of FGF-7 during intestinal damage (97). Mice lacking gamma delta T cells or lacking FGF-7 have more severe mucosal injury and a delay in intestinal repair during DSS-induced damage as compared to wild-type (97). A decrease in epithelial cell proliferation coincided with the delayed recovery phase in mice lacking gamma delta T cells or FGF-7 (97). Thus the gamma delta IEL plays a key effector role in the repair of the intestine via the production of keratinocyte growth factor. Taken together with the DETC model of wound repair, gamma delta T cells play key roles in epithelial homeostasis in the mouse, however this role is still controversial in the human (98, 99).

4.3. Lung IEL are involved in airway homeostasis

Lung gamma delta T cells reside in close contact with alveolar epithelial cells and have been implicated in protecting the environment and repairing damage caused by pathogens or chemicals (100). Damaging agents such as *Nocardia asteroides* and ozone have been used to examine both pathogenic and nonpathogenic sources of lung epithelial damage. Mice lacking gamma delta T cells are much more susceptible to death from *N. asteroides* infection and have areas of acute necrosis with few inflammatory cells in their lungs (100). Another pathogenic assault of the lung, influenza A, has been associated with increases in first the local gamma delta lung population, and then a Vgamma1 lymphoid population (101). Differences in the cytokine production of these two populations suggest that the resident lung gamma delta T cells may be pro-inflammatory, while the later Vgamma1 cells may help terminate the inflammation (102, 103).

Non-pathogenic insults of the lung have been examined to determine whether the gamma delta T cells are responding to the infectious agent or the self antigen expressed by damaged epithelial cells. Ozone causes nonpathogenic damage to a similar region of the lung as *N. asteroides*. Ozone-injured TCR delta knock-out mice had more necrotic cells than wild-type mice (100). The lack of a foreign antigen in these mice suggests that like DETC, lung IEL respond to the damaged epithelia itself. With both

N. asteroides and ozone assaults, there was a clear reduction in inflammatory cell infiltration. Since macrophages and neutrophils usually clear dead or dying cells, the collaboration of gamma delta T cells with inflammatory cells possibly via chemokine production may contribute to the susceptibility of TCR delta knock-out mice to lung injury.

There are other examples in which gamma delta T cells of the lung have been implicated in the maintenance of airway homeostasis and the hyperresponsiveness to antigen. Airway hyperresponsiveness can be found in diseases such as asthma. TCR delta knock-out mice exhibit decreased eosinophilia after OVA intranasal challenge with repeated OVA immunizations (104). There is also a lower level of IL-5 release in response to OVA challenge in knock-out mice as compared to mice with gamma delta T cells (104). In some studies this impairment in pulmonary inflammation was restored when IL-4 was administered *in vivo*, suggesting that either the IL-4-producing gamma delta T cells may trigger the initial events in eosinophil accumulation or the gamma delta T cells regulate alpha beta T cell production of IL-4 (104). Other studies have suggested that the gamma delta T cells actually maintain airway homeostasis and prevent airway hyperresponsiveness (105). In this case, mice lacking gamma delta T cells also had lower levels of inflammation in response to OVA, but developed higher airway hyperresponsiveness (105). Their data suggest that instead of targeting the inflammatory response of airway hyperresponsiveness, gamma delta T cells act downstream in a manner independent of alpha beta T cells or B cells (105). This mechanism of airway regulation may work along side the immunoregulatory role.

5. GAMMA DELTA T CELL FUNCTION IN HUMANS

The majority of gamma delta T cells in the human skin reside in the dermis, unlike murine DETC. Of the CD3+ cells in the epidermis, only approximately 20% are gamma delta cells (106-108). These epidermal gamma delta T cells do not display the dendritic morphology of murine DETC, further suggesting that a human counterpart to the murine DETC does not exist. However, similar to murine DETC, the delta repertoire in humans is somewhat restricted and different from the gamma delta cells in the blood and gut (109, 110). Furthermore, the epithelial gamma delta cells in human skin have been implicated in diseases such as cutaneous leishmaniasis and leprosy since they have elevated numbers in these patients and also have a restricted T cell repertoire in the areas of active disease (111, 112). Similar to the limited diversity of human skin gamma delta T cells, the human gut gamma delta IELs exhibit a restricted repertoire of Vdelta1 cells (113). Interestingly, the epithelia of the intestine of patients with Celiac or Crohn's disease have elevated numbers of gamma delta T cells (92, 114).

Functional studies of human gamma delta T cells have provided some information on the specificity of the various populations of T cells. For example gamma delta T cell recognition of several infectious agents, including mycobacteria (111, 115-117), have been reported. Interestingly, circulating gamma delta cells produce connective tissue growth factor (CTGF) possibly implicating them in the process of wound repair as well (118). Co-stimulation of the circulating gamma delta T cells with IL-15 and TGF-beta 1 increases CTGF levels substantially (118).

6. SUMMARY AND PERSPECTIVE

Until recently the roles that gamma delta T cells play in the immune system have not been well understood. Effector and regulatory roles have now been attributed to gamma delta T cells in a variety of diseases. The involvement of DETC in wound healing is an example in which both effector and regulatory roles are performed by one gamma delta T cell population. Research in both mice and humans has advanced our understanding of the molecules involved in the activation of gamma delta T cells. Studies on the biological roles of gamma delta T cells will help pave the way to therapies for epithelial tissues such as the skin or gut that are damaged, infected, or malignant.

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