Review

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2005;137:73–81
DOI: 10.1159/000085107

Published online: April 12, 2005

Epithelial Defence by γδ T Cells

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Key Words
Antimicrobial peptides · Chemokine receptors · Cytokines · γδ T cells · Intraepithelial lymphocytes

Abstract
γδ T cells constitute a separate lineage of T lymphocytes which differ from conventional αβ T cells with regard to T cell receptor (TCR) repertoire and tissue localization. In murine skin, γδ T cells expressing a canonical Vγ5 TCR are abundant and contribute as so-called dendritic epidermal T cells to local immune surveillance. In humans, major subsets of γδ T cells are recognized on the basis of their TCR Vδ usage. While Vδ2 cells dominate in the peripheral blood, Vδ1 cells are preferentially localized in mucosal tissue including the intestinal epithelia. In this article we summarize basic features of intraepithelial γδ T cells and discuss their possible role in epithelial defence.

Basic Features of γδ T Cells

In contrast to αβ T cells, γδ T cells do not recognize antigen in the context of classical major histocompatibility complex (MHC) molecules, in line with the absence of CD4 or CD8 coreceptors on most γδ T cells. γδ T cells also differ from αβ T cells with regard to the germ-line-encoded T cell receptor (TCR) repertoire [1]. While large numbers of variable α and β genes are available for selection during intrathymic T cell development, there are only six expressed human Vγ genes and a similarly small number of Vδ genes [2]. Nevertheless, the γδ TCR repertoire can be at least as diverse as the αβ TCR repertoire, due to the tremendous impact of mechanisms such as N nucleotide insertions during TCR gene rearrangement and usage of all three reading frames in the case of Dδ elements [3]. Interestingly, however, the expressed γδ TCR repertoire is highly biased, resulting in a preferential expression of some Vγ/Vδ genes in certain anatomical localizations. Thus, in the peripheral blood of adult humans there is a clear preponderance of γδ T cells expressing Vγ9 paired with Vδ2, which can represent 50–95% of all circulating γδ T cells ([4]; the nomenclature of the human Vγ/Vδ genes follows the nomenclature of Porcelli et al. [5]). While Vγ9/Vδ2 cells do not dominate early after birth, the shaping of the peripheral blood γδ TCR repertoire takes place during childhood when the relative expansion of Vγ9/Vδ2 T cells is thought to occur in response to exposure to environmental γδ T cell-stimulating microbial antigens [6]. In most healthy adults, other γδ T cell subsets are present only in low frequency in the blood. The second most frequent subset expresses the Vδ1 element which can be paired with any of the available Vγ chains. While Vδ1 cells are a minor population in the peripheral blood, they predominate at mucosal surfaces and are located within the epithelial layer of the small and large intestine [7, 8]. Interestingly, the TCR repertoire of intestinal Vδ1 T cells is highly restricted, as has been shown by sequencing of rearranged junctional regions of Vδ1 transcripts [9, 10]. Similarly to the situation with the Vγ9/Vδ2 T cells in the

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1018–2438/05/1371–0073$22.00/0

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peripheral blood, the Vδ1 T cells in the intestine are polyclonal at birth and display increasing junctional restriction with age [11]. Importantly, it was found that the Vδ1 TCR repertoires of circulating and intestinal Vδ1 T cells were clearly different in the same individual, suggesting that intraepithelial Vδ1 T cells fulfil functions linked to the recognition of locally displayed ligands or antigens [10]. Alterations in the peripheral blood Vδ1 TCR repertoire occur in certain conditions, notably in the context of viral infections. As an example, characteristic changes in the γδ TCR repertoire are observed in HIV-1 infection with a decrease in Vγ9/Vδ2 T cells being associated with a marked expansion of Vδ1 T cells [12–14]. It has been proposed that the expansion of peripheral blood Vδ1 γδ T cells in HIV-1-infected individuals might result from the recognition of ligands displayed on the polyclonally activated B lymphocytes [15]. An increase in circulating Vδ1 T cells was also found in renal allograft recipients developing a cytomegalovirus infection. Interestingly, substantial evidence indicated that the expanded Vδ1 T cells responded directly to viral glycoproteins in the absence of antigen-presenting cells [16].

Similar to the above-discussed compartimentalization of human γδ T cell subsets, there is also a strong correlation of the locally expressed γδ TCR repertoire and anatomical localization in the mouse. In contrast to humans, the mouse epidermis harbours large numbers of γδ T cells, commonly known as dendritic epidermal T cells (DETC) [17]. The γδ DETC appear to be of thymic origin and express a canonical Vγ5 TCR (nomenclature of Heilig and Tonegawa [18]), suggesting that they recognize an antigen restricted to the epidermis [19, 20]. At least some γδ T cells, notably intestinal intraepithelial lymphocytes (IEL) expressing Vγ7, are generated in the absence of a functional thymus [21]. A very recent study has extended these findings to demonstrate that γδ IEL can develop in athymic *nu/nu* mice lacking all lymph nodes including mesenteric lymph nodes, Peyer's patches, and the recently identified intestinal isolated lymphoid follicles [22], suggesting that at least some lymphoid cells can undergo TCR gene rearrangement in the gut mucosa.

Migration of γδ T Cells to Intestinal Mucosa and Skin

At least two mutually non-exclusive pathways orchestrate the ordered migration of lymphocyte subsets to defined target tissues, i.e. the interaction between adhesion molecules with their corresponding receptors, and the chemoattraction by locally produced chemokines of lymphocytes selectively expressing the adequate chemokine receptor [23]. Intestinal homing T lymphocytes express the chemokine receptor CCR9 and migrate in response to the chemokine CCL25/TECK which is produced in the small intestine [24]; as a consequence, CCR9 knockout mice have a severe deficiency in intraepithelial γδ T cells [25]. CCR9 is also expressed on intestinal homing human T cells and other mucosal lymphocytes [26]. While the expression of CCR9 on human γδ as compared to αβ T cells has not been analyzed before, we have recently investigated this issue on peripheral blood and intestinal IEL γδ T cells. We found very low expression of CCR9 on Vδ1 and Vδ2 blood γδ T cell subsets ex vivo and substantially higher induction on Vδ1 as compared to Vδ2 T cells upon TCR-dependent cellular activation. Furthermore, IEL γδ T cells strongly expressed CCR9 (as did αβ IEL) and maintained high level expression upon extended in vitro culture, in striking contrast to the αβ IEL. Thus, it appears that CCR9 also plays a crucial role for the intestinal localization of human γδ T cells.

The migration of T cells to the skin is governed by other chemokines, notably CCL17 (TARC) and CCL27 (CTACK) and their respective receptors CCR4 and CCR10 [27–29]. CCR4, however, is not selective for skin-homing lymphocytes but also governs migration of lymph node homing T cells in response to CCL22/MDC. In human peripheral blood lymphocytes, CCR4 is induced on Vδ2 γδ T cells upon TCR-dependent stimulation by bacterial phosphoantigens [30], and we also observed a strong CCR4 expression on IEL-derived human γδ T cell lines. Taken together, it appears that similarly to conventional αβ T cells, the coordinated expression of a selected set of chemokine receptors is correlated with the tissue localization of γδ T cells [31]. In addition to chemokines and their receptors, integrins are critically involved in this process. The integrin $\alpha_E\beta_7$ (CD103) is expressed by IEL and mediates lymphocyte adhesion to epithelial cells by interacting with the specific ligand E-cadherin [32]. Interestingly, the expression and the function of $\alpha_E\beta_7$ integrin is regulated by the chemokine CCL25, giving rise to a functionally important cross-talk with CCR9 on mucosa-seeking T cells [33].

Antigens Recognized by γδ T Cells

Conventional αβ T cells recognize processed peptides in the context of MHC class I (CD8+ T cells) or MHC class II molecules (CD4+ T cells). Instead, most γδ T cells

recognize different ligands, and usually in an MHC-non-restricted fashion [for reviews, see 1, 31]. The dominant subset of γδ T cells in human peripheral blood expressing Vγ9 paired with Vδ2 recognizes small microbial pyrophosphates derived from the bacterial non-mevalonate pathway of isoprenoid biosynthesis ('phosphoantigens') [34, 35]. Such phosphoantigens are produced by a variety of pathogenic bacteria, and rapidly induce proinflammatory cytokines including tumour necrosis factor-α and interferon-γ in Vγ9Vδ2 T cells [36, 37]. Human γδ T cells expressing Vδ1 are preferentially found among IEL as compared to peripheral blood. The Vδ1-encoded TCR recognizes MHC class I-related molecules (MICA/MICB) that are induced on epithelial cells by stress, suggesting that Vδ1 γδ T cells contribute to local immune surveillance [38–40]. Importantly, the stress-induced MICA antigens as well as some distantly related ULBP proteins are also ligands for NKG2D, an activating NK receptor expressed on γδ T cells, NK cells and some αβ T cells [41, 42]. Therefore, such MHC class I-related molecules which are induced on damaged ('stressed') epithelial cells can alert γδ IEL (notably Vδ1 cells) via multiple cell surface receptors. In addition, human Vδ1 γδ T cells have been found to recognize CD1 antigens [43] and cytomegalovirus proteins in the absence of MHC-dependent presentation [16].

In contrast to human Vγ9Vδ2 T cells, murine γδ T cells do not recognize bacterial phosphoantigens, due to a lack of homology in critically important TCR sequence residues. Therefore, it is impossible to use simple mouse models to address the pathophysiological significance of γδ T cell-mediated phosphoantigen recognition in vivo. Instead, murine γδ T cells have been found to recognize mycobacterial heat shock proteins, inducible MHC class Ib molecules T10/T22, poorly defined ligands on stressed keratinocytes and stressed intestinal epithelial cells [44–47], as well as a range of additional ligands [1, 31].

Effector Functions of γδ T Cells

The effector functions of activated γδ T cells resemble in many aspects those of conventional αβ T cells. Thus, γδ T cells produce cytokines and frequently exert potent cytotoxic effector function involving both perforin/granzyme and Fas/Fas ligand-dependent pathways [48, 49]. Although most γδ T cells seem to be primed towards the production of Th1-type cytokines, they have the intrinsic capacity to make Th2 cytokines including IL-4 if activated under appropriate Th2-driving conditions [50, 51]. However, a few seemingly specific effector functions of γδ T cells have been described. A striking example is the production of keratinocyte growth factor (KGF) by murine γδ DETC and γδ IEL [52]. Human γδ but not αβ T cells produce connective tissue growth factor (CTGF) which regulates wound healing and fibrinogenesis [53]. Moreover, human γδ T cells also produce fibroblast growth factor-9 (FGF-9) as well as KGF [54]. These observations are well in line with the notion that γδ T cells play an important role in epithelial repair mechanisms. In addition, several groups have searched for γδ T cell- and possibly localization-specific gene expression using transcriptional profiling and serial analysis of gene expression in murine γδ T cells [55–57]. These studies identified a variety of genes that are preferentially expressed by γδ IEL, and also revealed the rather unexpected overexpression of genes involved in lipid metabolism and cholesterol homeostasis [55].

Principles of Antimicrobial Epithelial Defence

In recent years it has emerged that naturally occurring antimicrobial peptides (AMP) play a central role in innate immune defence. Such AMP are produced by epithelial cells in the intestine, skin and elsewhere. Major classes of AMP comprise the defensins, the cathelicidins, and other peptide families including some RNases and members of the S100 protein family such as psoriasin [58–61]. Defensins are small polypeptides which exert their antimicrobial activity by permeabilization of the outer and inner bacterial cell membrane [62]. There are two major subfamilies, i.e. α- and β-defensins which share certain structural features but differ in other aspects. In humans, α-defensins are constitutively expressed and stored in granules in neutrophils, Paneth cells of the small intestine, and epithelial cells. In contrast, the expression of β-defensins in epithelial cells and the epidermis requires stimulation, e.g. by bacteria or bacterial products [58]. Some AMP require processing to exert bactericidal activity. Thus, the precursor of α-defensin is cleaved in murine Paneth cells in the small intestine by the matrix metalloprotease-7 (MMP-7 or matrilysin) [63–65]. The processing of the human cathelicidin hCAP-18 is mediated by a different protease, the serine protease proteinase 3. The cleavage of the C-terminal part by proteinase 3 liberates the antibacterial and cytotoxic peptide LL-37 [66]. LL-37 is a multifunctional molecule which, in addition to its bactericidal activity, exerts multiple modulatory effects on innate immune responses. In particular, LL-37 modulates gene expression in monocytes, induces IL-1β processing

and release, and influences the differentiation of dendritic cells and dendritic cell-induced T helper cell polarization [67–69]. At the molecular level, at least some of these effects are linked to the activation of mitogen-activated protein kinases ERK1/2 and p38 [70, 71]. Remarkably, the production of cathelicidin/LL-37 is not restricted to epithelial cells and keratinocytes but is also observed in neutrophils, monocytes and mast cells [72–74]. Most interestingly, LL-37 expression at the mRNA and protein level was also found in human γδ T cells [73], well in line with our own observations. This raises the possibility that γδ IEL could directly contribute to antimicrobial defence by producing certain AMP (see below).

Defensins are not typically produced by immune cells. α-defensins HNP1–3, however, are expressed by some NK and T cells [73], and are induced in NK cells upon stimulation with bacterial products such as flagellin [75]. In addition, Duits et al. [76] observed expression of β-defensin-1 (hBD-1) and hBD-2 in human blood monocytes and alveolar macrophages, supporting the idea that monocyte and/or dendritic cell-derived β-defensins might contribute to orchestrating an immune response by attracting T lymphocytes via the β-defensin receptor CCR6 [76, 77]. In this regard, it is of interest that we recently observed the strong expression of CCR6 on IEL-derived but not on peripheral blood-derived γδ T cell lines, suggesting a structural basis for a cross-talk between epithelial cells and γδ IEL. In addition, we also found expression of hBD-2 mRNA by RT-PCR in a proportion of the analyzed human γδ T cell lines. A systematic analysis of defensin expression in IEL-derived γδ T cells is under way in our laboratory.

Regulatory Role of γδ T Cells in the Skin

As mentioned above, the mouse skin harbours large numbers of γδ DETC. These DETC express a canonical Vγ5Vδ1 TCR with identical junctional sequences and are activated by coculture with stressed keratinocytes. The activation of DETC by stressed keratinocytes requires cell-cell contact, suggesting TCR-mediated recognition of self antigen [46]. In their recent experiments, Jameson et al. [78] isolated low molecular weight fractions from stressed murine keratinocytes which activated γδ DETC but not γδ T cells from other tissues expressing different TCR. Further work is needed, however, to precisely identify the antigen seen by the DETC TCR. In any case, it appears that the γδ DETC present in murine skin play a non-redundant role in the local surveillance of stressed or damaged keratinocytes. TCRδ$^{-/-}$ mice lacking all γδ T cells still have some DETC which express a polyclonal αβ TCR instead of the canonical γδ TCR. Interestingly, these αβ DETC can be activated by mitogen or anti-CD3 antibodies but do not respond to keratinocyte damage, indicating the unique role of the γδ TCR expressed on DETC [79]. The close contact of epidermal keratinocytes with γδ DETC suggests a critical function of the DETC in the process of wound healing. In fact, it has been found that γδ DETC produce KGF-1/FGF-7 in response to contact with damaged keratinocytes, which supports keratinocyte proliferation and thus wound repair [80]. As shown in a skin organ culture system, keratinocytes proliferated at the wound site of wild-type skin but not skin from TCRδ$^{-/-}$ mice. In addition to KGF-1/FGF-7, FGF-10 was also expressed in γδ DETC and thus could also contribute to early keratinocyte proliferation [81]. Taken together, these results indicate that γδ T cells play an important role in the process of wound repair in the mouse skin by producing KGF. In addition, γδ T cells also regulate local cutaneous inflammatory reactions. This was nicely shown in a study by Giradi et al. [82] where the authors investigated spontaneous dermatitis occurring in TCRδ$^{-/-}$ mice of different genetic backgrounds. While both NOD.δ$^{-/-}$ and FVB.δ$^{-/-}$ mice spontaneously developed localized chronic dermatitis, C57BL/6.δ$^{-/-}$ mice did not. The dermatitis was associated with the accumulation of large numbers of αβ T cells in the skin, suggesting that γδ DETC are important in controlling migration of inflammatory αβ T cells into the skin. Other reports have shown that local γδ T cells also regulate contact hypersensitivity. TCRδ$^{-/-}$ mice showed increased contact hypersensitivity responses, due to uncontrolled activity of hapten-specific CD8+ T cells [83]. Together with many additional studies not cited here for lack of space, these results underline the idea that γδ T cells have important immunoregulatory functions, especially when located in epithelial and mucosal tissue [84].

γδ T cells also contribute to the control of cutaneous malignancy in mice. As shown by Girardi et al. [85], mice lacking γδ T cells were much more susceptible to developing cutaneous malignancy in a two-stage tumour initiation and promotion model. After exposure to carcinogen, the skin cells expressed MHC class I-related molecules, Rae-1 and H60 molecules, which are ligands for NKGD2 receptors on local γδ T cells in wild-type mice. In this model of chemically induced skin cancer development, γδ T cells are strongly protective, while αβ T cells can contribute to tumour progression [86]. Together, this is clear evidence that local γδ T cells fulfil an important

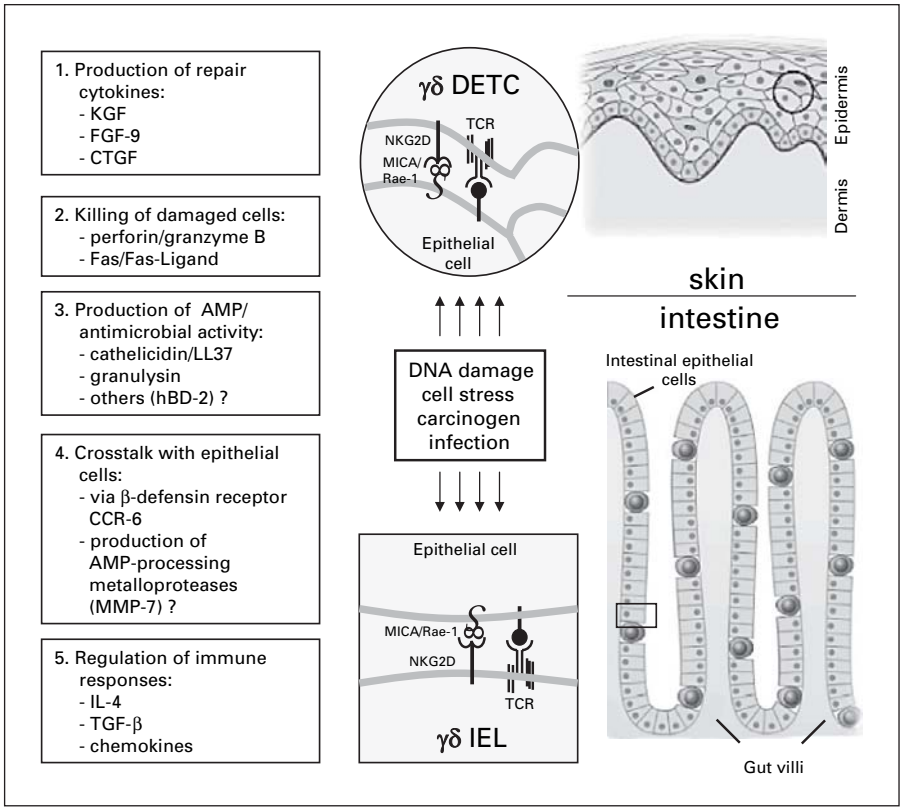

Fig. 1. Possible functions of intraepithelial γδ T cells. The DETC in the mouse skin are γδ T cells expressing a canonical T cell receptor (Vγ5). Although intraepithelial γδ T cells in the gut mucosa are somewhat more diverse, they preferentially express Vγ7 in the mouse and Vδ1 in humans. Intraepithelial γδ T cells can be activated via two pathways: (1) TCR-dependent ligand recognition and (2) binding of stress-induced MHC class I-related molecules on epithelial cells (MICA/B, ULBP in humans, Rae-1/H60 in the mouse) to activating NKG2D receptors expressed on the γδ T cells. Possible effector functions of the activated γδ T cells are listed in the boxes on the left hand side.

role in downregulating epithelial malignancy. Other potential functions of γδ DETC, such as the possible production of AMP, have not yet been investigated.

Function of γδ T Cells in the Intestinal Mucosa

γδ T cells constitute a major proportion of the IEL population in the intestinal mucosa. It appears that IEL γδ T cells also exert a non-redundant function in the mucosal tissue. Chen et al. [87] used the dextran sodium sulphate (DSS)-induced mouse colitis model to address this issue. They noted an accumulation of large numbers of γδ but not of αβ T cells at the sites of DSS-induced epithelial cell damage. More severe colitis and delayed tissue repair were observed in TCRδ$^{-/-}$ mice lacking all γδ T cells. Again, KGF was identified as the γδ IEL-derived growth factor that promoted localized epithelial cell proliferation and thus tissue repair following DSS-induced colitis. Very similar results were obtained by Yang et al. [52] in a different in vivo model, i.e. villus atrophy induced by total parenteral nutrition and villous hypertrophy resulting from a short bowel syndrome. The former was associated with a downregulation of γδ IEL-derived KGF, whereas upregulation was observed in the latter case. This is additional evidence that IEL γδ T cells critically control epithelial cell growth through the production of appropriate growth factors. Moreover, a recent study points to an important role of the transcription factor interferon regulatory factor-1 (IRF-1) in the control of intestinal γδ T cell homeostasis. As shown by Siegmund et al. [88], IRF-1 knockout mice developed a dramatically more severe colitis and showed higher mortality than wild-type mice following DSS treatment. Interestingly, the IRF-1$^{-/-}$ mice had much fewer γδ IEL (<50%) as compared to wild-type mice. While the reduced number of γδ IEL might contribute to the development of colitis in the IRF-1$^{-/-}$ mice, it is clear that other mechanisms are also involved, such as the strongly reduced production of the IL-18 antagonizing IL-18 binding protein in these mice [88].

On the basis of their distribution and cluster formation with epithelial cells, it is likely that γδ IEL also play a role in the local surveillance of the human gut epithelium [89]. The function of intestinal γδ T cells in inflammatory bowel disease in humans is not completely understood. Reports on increased numbers of γδ T cells in the inflamed mucosa [90] contrast with contradictory studies [see 8,

91]. Several reports indicate an accumulation and clonal expansion of γδ T cells in the inflamed mucosa of patients with Crohn's disease [92, 93] as well as increased numbers of Vδ1 γδ T cells also in the peripheral blood [91, 94]. However, the molecular analysis of the TCRδ repertoire revealed a lack of dominant clones in the inflamed mucosa, but distinct repertoires in the intestine as compared to blood [8, 95]. Human γδ IEL frequently express Vδ1 which recognizes the stress-inducible MICA antigens. This allows Vδ1 γδ T cells to control epithelial integrity by eliminating stressed or damaged cells, similarly to the above-described situation in the mouse skin. In addition to γδ T cells, other lymphocyte populations including the NKT cells expressing invariant Vα24Vβ11 TCR are likely to contribute to intestinal immunity and epithelial defence [96]. Presently, it is not known whether γδ IEL contribute to antimicrobial defence by producing AMP. Our preliminary results would indicate that some γδ T cells express certain AMP. In addition, we found that γδ T cells can express matrilysin (MMP-7), suggesting that γδ T cells might contribute to epithelial homeostasis via production of this metalloprotease known to be required for α-defensin processing in Paneth cells of the mouse gut [63, 64].

Concluding Remarks

Local γδ T cells can be activated by diverse stimuli such as stress-induced self antigens expressed on epithelial cells/keratinocytes due to infection, DNA damage, or carcinogen contact. As summarized in figure 1, these γδ T cells can among other things (1) assist wound healing by providing keratinocyte and fibroblast growth factors, (2) kill unwanted damaged cells via perforin/granzyme and/or Fas-Fas ligand-dependent pathways, (3) potentially mediate direct antimicrobial activity by producing certain AMP, (4) possibly communicate with epithelial cells via CCR6, and (5) exert immunoregulatory activity through the release of cytokines and chemokines [83, 84, 97–100]. Therefore, γδ IEL located in the skin or in the intestinal mucosa constitute an integral part of epithelial defence mechanisms.

Acknowledgements

Our work cited in this paper was supported by the Deutsche Forschungsgemeinschaft (SFB 617 project A16). This work forms part of the PhD thesis of Lothar Marischen.

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