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Invariant Vα7.2-Jα33 TCR is expressed in human kidney and brain tumors indicating infiltration by mucosal-associated invariant T (MAIT) cells

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Abstract

The anti-tumor response of human invariant NKT (NKT) cells is well established. A novel T cell subset, mucosal-associated invariant T (MAIT) cells, possesses similar regulatory properties to NKT cells in autoimmune models and disease. Here, we examined the clonality of four T cell subsets expressing invariant αTCR, including Vα7.2-Jα33 of MAIT cells, in 19 kidney and brain tumors. The MAIT clonotype was identified and co-expressed with NKT clonotype in half of the tumors. In contrast, two other invariant T cell clonotypes (Vα4 and Vα19) were not present in tumors. Such tumors also expressed Vβ2 and Vβ13, the restricted TCRβ chain of MAIT cells and the antigen-presenting molecule MR1. A high percentage of infiltrating T cells was CD8+ and expressed HLA-DR suggesting activation. Although the MAIT αTCR was identified in both peripheral CD56+ and CD56− subsets, infiltrating lymphocytes were CD56 negative. The clonal presence of MAIT cells in tumors correlated with the expression of pro-inflammatory cytokines but no IL-4, IL-5 and IL-10, suggesting that a pro-inflammatory subset of human MAIT cells may exist. Our data imply that a CD56− subset of MAIT cells may participate in tumor immune responses similarly to NKT cells.

Introduction

The heterogeneous T cell repertoire of mainstream lymphocytes is generated by random recombination of V, D and J segments and junctional deletion and insertion of nucleotides. In contrast, innate lymphocytes are characterized by limited repertoire diversity. The best-characterized invariant αβT cell population, NKT cells, was identified in the early 90s in humans (1, 2). NKT cells express the invariant Vα24-JαQ CDR3 and reside mostly in the CD4−CD8− (double negative (DN)) and CD4+ cell subsets (1, 2). The CD1d recognition of DN Vα24 T cells and the restricted Vβ usage defined this subset as the human analog of murine NKT cells (3, 4). Emerging data indicate the functional diversity of human NKT cells and their involvement in tumor immunity and autoimmunity (5–8).

Along with the identification of Vα24 NKT cells, another DN T cell population expressing an invariant Vα7.2-Jα33 TCR has been described (1). Homologous TCR sequences displaying the same CDR3 length could be identified in bovine and murine DN T cell subsets (Vα19.1-Jα26), suggesting an important physiological function (9). Out of the two *TCAV7* genes, most of the conserved Vα7+ clones utilize the *AV7.2* gene segment in humans (10–12). In addition to the conserved CDR3α, mucosal-associated invariant T (MAIT) cells express a restricted Vβ2 and Vβ13 driven by the selecting antigen (9, 10). The preferential location of invariant Vα7.2-Jα33 T cells is the gut lamina propria; hence, the name MAIT cells has been recently suggested (13). Nevertheless, MAIT cells are also present in the peripheral blood at a similar frequency to Vα24 NKT cells (0.1–0.2%) and constitute about up to 15% of DN T cells (10). Vα7.2-Jα33 MAIT cells are selected by a non-classical MHC class Ib molecule, MR1 encoded by chromosome 1, similarly to CD1d. Several lines of evidence suggest that MR1 presents ligands to MAIT cells, possibly glycolipids, similarly to NKT cells (13–16).

Recent data suggest that MAIT cells are similar to NKT cells in a number of aspects: both use a semi-invariant TCR, recognize glycolipids and are activated in a co-receptor-independent manner, selected and restricted by a monomorphic class I-like molecule and characterized by a natural memory phenotype, suggesting a high-avidity interaction with

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self (8, 9, 12, 13, 16). In NKT cell-deficient mice, NK1.1+ hybridomas express the Vα19.1-Jα26+ TCR homologous to human Vα7.2-Jα33 (17). In Vα19.1-Jα26 TCR transgenic mice, the transgenic T cells also express NK1.1, the surface molecule of NKT cells (18).

So far, data about function of human MAIT cells are very limited, partly due to the absence of clonotypic antibodies. In humans, the invariant TCR of MAIT cells was shown to be expressed in autoimmune lesions of the central nervous system (CNS) and peripheral nervous system (PNS), which correlated with the expression of IL-4, suggesting an anti-inflammatory role and regulating autoimmune response similar to mice (12, 18, 19). However, a functional diversity similar to NKT cells has been very recently indicated in mice (20).

In addition to NKT and MAIT cells, two other T cell subsets expressing an invariant α chain have been suggested. These additional T cell populations express a Vα4-Jα29 and Vα19-Jα48 TCR, respectively (11).

Here, we examined expression of the invariant TCRs of the four human innate T cells in tumors using single-strand conformation polymorphism (SSCP) clonotype analysis. This method has been used to detect clonal dominance and expansion of T cell clones in different T cell subsets, including NKT and MAIT in autoimmune lesions of the nervous system (12, 19, 21). The selective absence of the invariant Vα24-JαQ TCR of invariant NKT (NKT) cells in CNS plaques of patients with multiple sclerosis (MS) was previously observed, while conventional Vα24 TCRs and invariant TCR of MAIT cells were present (12, 19). To partly examine whether absence of NKT cells in CNS plaques might be related to the CNS compartment or is specific to MS, here we examined tumors inside and outside the CNS, i.e. malignant brain tumors and kidney cancers. In tumors characterized by clonal presence of MAIT cells, we also examined the expression of CD56, pro- and anti-inflammatory cytokines, restricted TCR-Vβ chains and MR1.

Methods

The study protocol was approved by the Regional Local Ethics Committee. Patients or representatives gave written permission to perform all procedures only performed due to medical purposes supporting diagnostic and therapeutic decisions.

Isolation of messenger RNA and synthesis of cDNA

Nineteen tumor samples were obtained by biopsy or during operation (11 clear cell kidney cancer, 6 glioblastoma and 2 malignant meningioma) and immediately snap frozen or processed. None of the patients was treated with immunosuppressants or irradiation before or at the time of sampling. Messenger RNA (mRNA) was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany). The air-dried pellet was re-suspended in 20 μl of RNAse-free water and used for cDNA synthesis by First-Strand cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden, or Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) using oligo-dT as primer.

SSCP analysis

Briefly, mRNA was isolated from tumor tissues, and cDNA of αCDR3 regions was amplified by reverse transcription (RT)–PCR with Vα- and Cα-specific primers. Primers and probes were designed based on the previously published sequences (12, 21). In brief, 1 μl of the diluted cDNA was used for each PCR with one of the TCR-Vα-specific primers and a Cα-specific primer. Sense primers specific for Vα7.2 (GTCGGTCTAAAGGGTACAGT) and Vα19 (GCCACAATAAACATACAGGA) were used in conjunction with the same anti-sense Cα primer (CAGCTGAGAGACTCTAAAT). Sense primer for Vα4 (ACAGAATGGCCTCTCTGG) was used with another anti-sense Cα primer (ATCGGTGAATAGGCAGACAG). To detect Vα24-JαQ-invariant human NKT cells, RT–PCR was performed as described previously with Vα24-specific sense primer (ACACAAAGTCGAACGGAAG) and Cα-specific anti-sense primer (GATTTAGAGTCTCTCAGCTG) (21). cDNA synthesized from mRNA of biopsy samples was amplified for 40 cycles, diluted (1:3) and heat denaturated.

Four microliters of the diluted samples were electrophoresed in non-denaturing 4% polyacrylamide gel. DNA was transfered to Immobilon-S (Millipore Intertech, Bedford, MA, USA) and hybridized with a biotinylated Cα-specific, Jα-specific or α-CDR3-specific clonotypic internal probe. Hybridization with a Cα probe detects all amplified CDR3 sequences (clonotypes) representing distinct T cell clones. In samples with a heterogeneous T cell population, the SSCP pattern is characterized by a smear, while CDR3 sequences of the dominant or expanding clones appear as distinct bands reflecting clonality of the repertoire (12, 19, 21). The probes were as follows: Cα (AAATATCCAGAACCCTGACCCTGCCGTGTACC), Jα29 (CTCTTGTCTTTGGAAAGGGCACAAGACTTTCTGT), Jα33 (TATCAGTTAATCTGGGGCGCTGGGACCAAGCT), Jα48 (ATTAACCTTTGGGACTGGAACAAGACTCACCATC) and Vα24inv, clonotypic (TGTGTGGTGAGCGACAGAGGCTCAACCCTG).

DNA was visualized by subsequent incubations with streptavidin, biotinylated alkaline phosphatase and a chemiluminescent substrate system (Phototope™ Detection Kit, New England Biolabs, Inc., MA, USA).

RT–PCR

cDNAs for human β2-microglobulin, IL-4 and IFN-γ were amplified by RT–PCR as described previously (12, 21). In brief, 1 μl of cDNA was used in 25 μl PCR mixture using PCR Master Mix (Promega, Madison, WI, USA). Similar strategies were used for amplification of other cytokines and MR1 mRNA. The primer sequences and annealing temperatures are indicated in Table 1. cDNA was amplified in GeneAmp 2700 amplifier (Applied Biosystems) using 39 cycles. The clonotypic RT–PCR for the detection of the invariant MAIT TCRα chain was performed as described previously, by using Vα7.2-specific sense and clonotypic invariant Vα7.2-Jα33 anti-sense primers (Table 1) (12).

Flow cytometry and sorting of lymphocyte populations

Thirty milliliter heparinized peripheral blood was obtained from patients with clear cell kidney cancer, brain tumors and from healthy subjects. PBMCs were isolated on Ficoll-Paque gradient (GE Healthcare, Uppsala, Sweden) by density gradient centrifugation. Fresh, unfixed tumor tissue blocks (~1 cm^3) obtained by nephrectomy were immediately sampled in the

Table 1. Primer sequences and annealing temperature

	Primer sequences (5′–3′)	Annealing temperature (°C)
TNF-α	F: caatgccctcctggccaat; R: tcggcaaagtcgagatagtc	58
IL-17	F: aatctccaccgcaatgagga; R: acgttcccatcagcgttgat	58
IFN-γ	F: atgtagcggataatggaactc; R: aacttgacattcatgtcttcc	58
IL-12	F: attctcggcaggtggaggt; R: gcagaatgtcagggagaagt	58
IL-4	F: actgcaaatcgacacctatta; R: atggtggctgtagaactgc	58
IL-5	F: gcttctgcatttgagtttgctagct; R: tggccgtcaatgtatttctttattaag	60
IL-10	F: gaaccaagacccagacatc; R: cattcttcacctgctccac	58
MR1	F: tgggagaggtacactcagc; R: agccacattatctacagcca	58
Vβ13A	F: gtatcgacaagacccaggc	62
Vβ13B	F: ggctcatccattattcaaatac	60
Vβ2	F: tcatcaaccatgcaagcctg	60
Cβ	R: gcttctgatggctcaaacac	
Vα7.2-Jα33	F: gtcggtctaaagggtacagt; R: tgatagttgctatctctcac	58
β2-Microglobulin	F: aagatgagtatgcctgccgtg; R: cggcatcttcaaacctccat	58

operation theater from the tumor–kidney border region or from brain tumors, kept on a humid atmosphere at +4°C and transported within an hour time to the flow cytometry laboratory. Tumor tissues were cut into cubic millimeter pieces by a sharp sterile surgical knife, immersed in 2 ml, pH 7.4, PBS and pushed gently through a 100-μm microfilter (Millpore). The cell counts of the filtered tumor cell suspensions were measured by a 'routine' laboratory volumetric hemocytometer (CellCyn3700, Abbott, USA) and adjusted to 5×10^6 cells per ml in PBS. After washing in PBS, 1×10^6 PBMCs and 5×10^6 tumor cells were incubated for 30 min at room temperature with different dual or triple combinations of the following mAbs: FITC-conjugated anti-CD3 and anti-CD4; PE-conjugated anti-CD8, anti-CD4, anti-HLA-DR and allophycoerythrin- or perCP-conjugated anti-CD56 and anti-CD45 (all antibodies from Becton Dickinson, San Diego, CA, USA). At least 50 000 cells were analyzed using a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, Erembodegem, Belgium) after single gating on lymphoid cells for all mAb combinations. Tumor cells were gated out from the tumor-infiltrating lymphocyte populations by their large forward/side scatter and $CD45^{dim}$ staining characteristics. The percentages of positive cells were calculated using CellQuest software (Becton Dickinson).

CD56+ cells were positively selected with CD56 MicroBeads on a MACS Cell Separation Column according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany).

Immunohistochemistry

After endogenous peroxidase blocking, slides were incubated with antibodies against CD3 (Dako, prediluted), CD4 (Labvision, 1:40), CD8 (Labvision, 1:50) and CD56 (Novocastra, 1:50). The antibodies were visualized in an automated immunostainer (Ventana Medical System).

Results

Detection of the invariant Vα24-JαQ TCR sequence in biopsy samples of kidney cancer and brain tumors

In order to investigate the presence of human NKT cells in tumor tissues, we applied SSCP clonality assay (21). We could detect Vα24 mRNA in all kidney cancers and six out of eight CNS tumors. Control kidney and brain samples were negative for the Vα24+ TCR (data not shown). The number of infiltrating clonotypes varied between two and nine per sample in kidney cancer, and there was a great variation in the number and dominancy of certain clonotypes. Furthermore, a clonotype in the same position was identified in different samples indicating the presence of a shared, invariant αCDR3 (Fig. 1). Indeed, hybridization with the invariant clonotypic probe revealed a single band. The invariant clonotype was present in 11 out of 19 cancer tissues (7 out of 11 kidney and 4 out of 8 brain) (Fig. 1).

Detection of the invariant Vα7.2-Jα33 TCR sequence in biopsy samples of kidney cancer and brain tumors

The expression of Vα7.2+ clonotypes and the invariant Vα7.2-Jα33 MAIT TCR was examined by previously established SSCP method, similar to the detection of NKT TCR (12, 19). Vα7.2+ clonotypes were detected in 8 out of 11 kidney cancer and 6 out of 8 brain tumors. Similar to the Vα24+ T cell population, CNS samples were characterized by smear with a few, less dominant clonotypes compared with kidney cancers when the membrane was hybridized with a Cα-specific probe. A common clonotype representing the Vα7.2-Jα33-invariant αCDR3 was found in the Vα7.2 repertoire confirmed also by hybridization with a Jα33-specific probe (12). Invariant clonotypes could be detected in 14 out of 19 cancer samples (8 out of 11 kidney and 6 out of 8 brain tumors) (Fig. 2). In both kidney and brain tumors, the MAIT clonotype was more dominant than in the peripheral blood (PB) (Fig. 2, lower panel).

Search for invariant Vα4+ and Vα19+ TCR sequences in tumors

In addition to MAIT and NKT cells, Vα4-Jα29+ and Vα19-Jα48+ T cells have been shown to express non-canonical αCDR3s (11). To investigate their presence among tumor-infiltrating lymphocytes, we established SSCP clonotypic assays.

Vα19+ mRNA was detected in 5 out of 11 kidney cancers (45%) but in none of the brain tumors. Similarly, Vα4+ mRNA could be amplified from six kidney cancers (54%), but was not present in brain tumors. The infiltrating Vα4+ repertoire

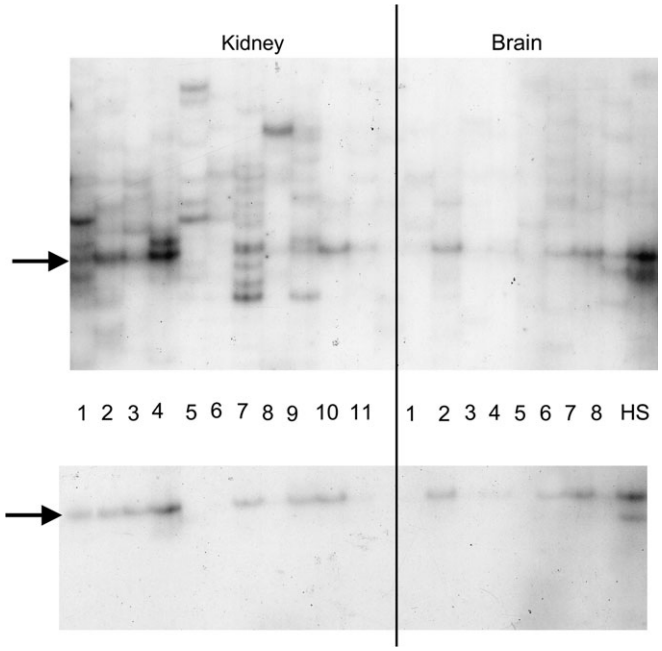

Fig. 1. Clonality of the Vα24+ T cell repertoire and presence of the invariant Vα24-JαQ clonotype representing NKT cells in kidney and brain tumors. Tumor tissues obtained from 11 patients with clear cell kidney cancer (left panel) and eight patients with brain tumor (right panel) were examined by RT–PCR SSCP clonotypic analysis. Amplified Vα24+ CDR3 was hybridized with a Cα probe (upper panel) and an invariant, clonotypic CDR3-specific probe (lower panel). HS indicates a lane for PBMC from a healthy subject. Arrow shows the position of the clonotype representing the invariant Vα24-JαQ CDR3 in several samples indicating the presence of NKT cells.

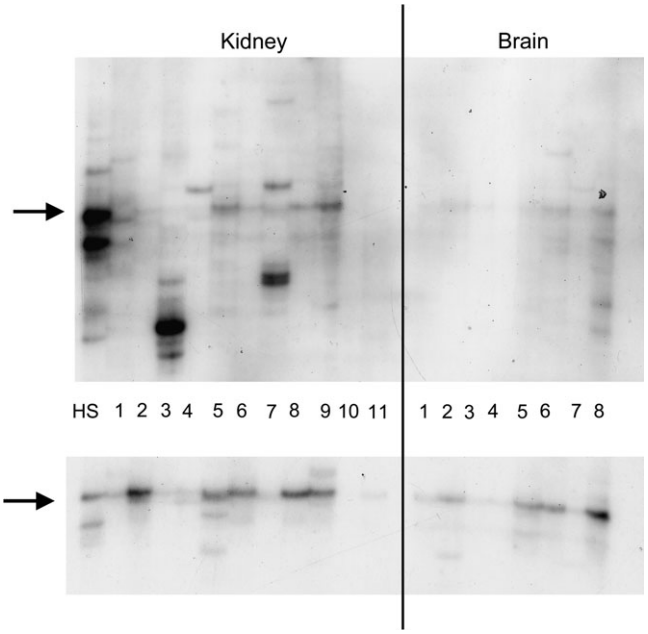

Fig. 2. Clonality of the Vα7.2+ T cell repertoire and presence of the invariant Vα7.2-Jα33 clonotype representing MAIT cells in kidney and brain tumors. Tumor tissues obtained from 11 patients with clear cell kidney cancer (left panel) and eight patients with brain tumor (right panel) were examined by RT–PCR SSCP clonotypic analysis. Amplified Vα7.2+ CDR3 was hybridized with a Cα probe (upper panel) and a Jα33-specific probe (lower panel). HS indicates a lane for PBMC from a healthy subject. Arrow shows the position of the clonotype representing the invariant Vα7.2-Jα33 CDR3 in several samples indicating the presence of MAIT cells.

was very restricted in kidney cancers indicated by the presence of a few, well-demarcated clonotypes. In contrast, the Vα19+ T cells expressed heterogeneous αCDR3 (Fig. 3). We were not able to identify shared Vα4+ or Vα19+ clonotypes hybridizing the samples with Cα- or Jα-specific probes (Fig. 3), suggesting that presence of MAIT and NKT clonotypes in tumors may not be accidental.

Detection of Vβ2 and Vβ13 TCRβ chains expressed by MAIT cells in kidney and brain tumors

Beside the invariant Vα7.2-Jα33 TCRα chain, MAIT cells are characterized by a restricted Vβ2 and Vβ13 TCR usage (9, 10). We examined the expression of these TCRβ chains by RT–PCR. In kidney cancers, all but one sample expressed Vβ2 and Vβ13 mRNA, respectively (Fig. 6). In addition, all the eight samples expressing the MAIT clonotype were positive for both β chains. In contrast, expression of these β chains was more limited in brain tumors: Vβ2 and Vβ13 sequences could be detected in five samples, and half of the brain tumors expressing the invariant MAIT αTCR did not express Vβ2 and Vβ13.

T cell subtypes in tumors expressing the invariant MAIT αTCR

To further characterize tumor samples expressing invariant αTCR and restricted TCRβ chains of MAIT cells, tissues were stained with anti-CD3, anti-CD4, anti-CD8 and anti-CD56

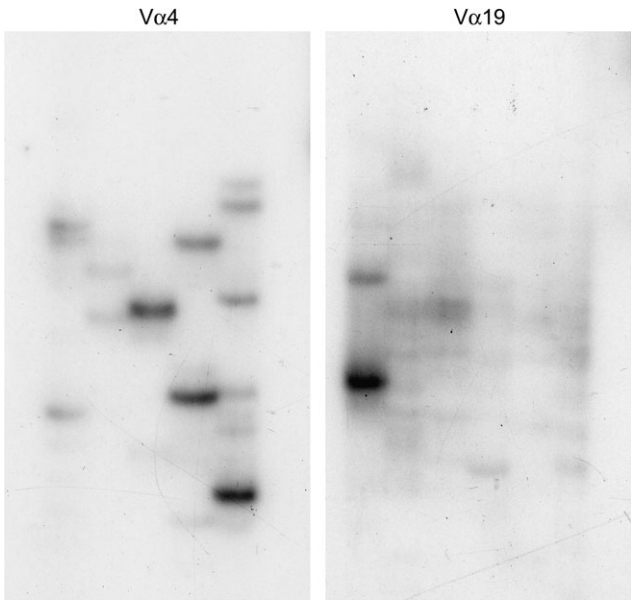

Fig. 3. Clonality of the Vα4+ and Vα19+ T cell repertoire in clear cell kidney cancer. Tumor tissues expressing Vα4+ and Vα19+ TCR were further analyzed by SSCP clonality analysis using Cα-specific probe to detect clonotypes with invariant TCR. No shared clonotypes are present in five kidney cancers positive for Vα4+ TCR (left panel) and seven kidney cancers expressing Vα19+ TCR (right panel)

antibodies. All kidney and brain tissues were positive for CD3+ lymphocytes, as expected. Only a few lymphocytes expressed CD4 in contrast to CD8 co-receptor (Fig. 4). FACS staining of infiltrating lymphocytes confirmed that HLA-DR+CD8low T cells were the major subset in tumors expressing MAIT TCRs (Fig. 4).

However, infiltrating T lymphocytes did not express CD56 in either tumor, despite of previous data suggesting expression of this molecule by NKT and MAIT cells as well (4, 9) (Fig. 5).

Detection of the invariant MAIT αTCR in CD56 subsets of PBMC obtained from patients with kidney and brain tumors

The absence of CD56+ T cells in tumors expressing MAIT and NKT TCR was unexpected (Fig. 5). Therefore, we examined the expression of the invariant Vα7.2-Jα33 TCRα chain in peripheral CD56+ and CD56− T cell subsets isolated from PB of five patients with kidney cancer, five patients with brain tumors and five healthy subjects. CD56+ and CD56− subsets were isolated by MACS from PB, and the invariant MAIT TCR was amplified by clonotypic RT–PCR. Both CD56+ and CD56− subsets obtained from patients and healthy controls expressed MAIT TCR (Fig. 5).

Cytokine and MR1 expression in kidney and brain tumors infiltrated by MAIT and NKT cells

While the functional heterogeneity of NKT cells is well established, MAIT cells are regarded as a T cell population producing primarily T_h2 cytokines (9, 12, 17, 18). This view has been recently challenged in mice (20). Therefore, we attempted to examine the cytokine environment in tumors expressing MAIT and NKT TCR (Fig. 6). Tumor samples were examined for the presence of MAIT and NKT clonotypes by RT–PCR SSCP and correlated with pro/anti-inflammatory cytokine expression examined by RT–PCR in the same samples (Tables 2 and 3).

The majority of tumors expressed pro-inflammatory (T_h1 and T_h17) cytokines. IL-12 and tumor necrosis factor (TNF)-α mRNA were equally well represented in both kidney and brain tumors. In contrast, only a single brain tumor expressed IL-17 and none was positive for IFN-γ mRNA, although both cytokines were abundantly expressed in kidney cancer (Tables 2 and 3).

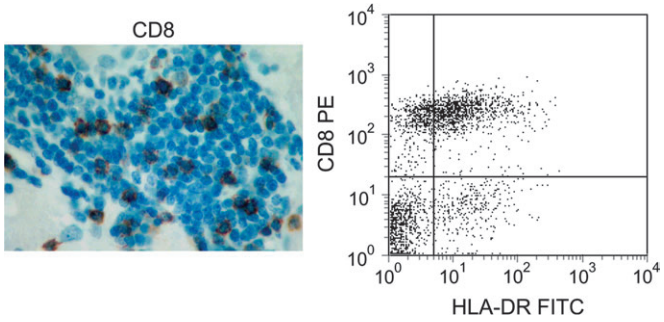

Fig. 4. Phenotype of infiltrating T lymphocytes in tumor tissues expressing the invariant MAIT αTCR. Surface expression of CD8 by immunohistochemistry (×400) and CD8/HLA-DR ontumor-infiltrating lymphocytes analyzed by flow cytometry is shown in a representative sample.

A similar bias was observed in the case of anti-inflammatory cytokines: only IL-10 mRNA was detected in abundance in kidney cancers. Three kidney cancers were also positive for IL-4 and IL-5 mRNA, but these cytokine messages were not detected in brain tumors at all. In addition, three of the kidney cancers and three of the brain tumors expressed only pro-inflammatory cytokines despite the presence of the invariant MAIT TCR (Tables 2 and 3).

To examine whether infiltrating MAIT cells may be locally activated through antigen recognition, expression of MR1 was also checked (Tables 2 and 3 and Fig. 6). All but one kidney cancer and four out of six brain tumors expressing MAIT TCR were positive for MR1 mRNA. In contrast, we identified 4 out of 19 tumors, which expressed MR1 but no MAIT invariant TCR.

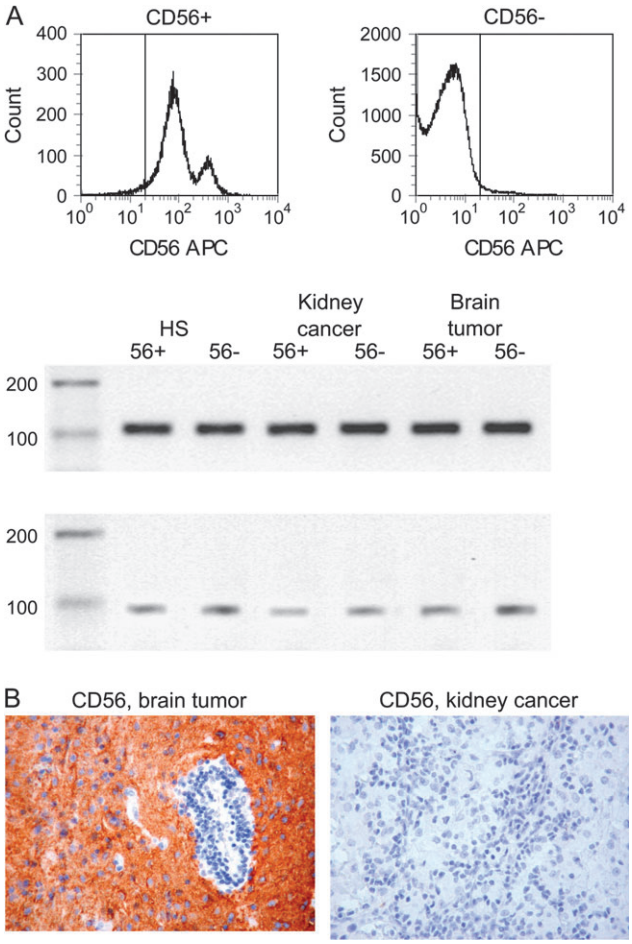

Fig. 5. Expression of CD56 in tumors expressing MAIT αTCR and presence of MAIT αTCR in peripheral CD56+ and CD56− lymphocyte subsets obtained from cancer patients and controls. (A) CD56+ and CD56− lymphocyte subsets were isolated from the peripheral blood of healthy controls, patients with clear cell kidney cancer and patients with brain tumors by MACS. Purity of the sorted population was checked by flow cytometry. Expression of β2-microglobulin (upper panel) and the invariant MAIT αTCR by a clonotypic RT–PCR (lower panel) was examined in the CD56+ and CD56− subsets. (B) Clear cell kidney cancer and brain tumors expressing the MAIT αTCR were examined for the expression of CD56 by immunohistochemistry. The positive staining of CNS served as positive control (brown, ×200).

Discussion

The role of NKT cells in tumor immunity is well established (5, 22, 23). In contrast, other innate T lymphocytes expressing canonical αβTCR have not been examined. A novel innate T cell subset, MAIT cells, is particularly interesting since phenotypic and functional similarities to NKT cells have been already suggested (8, 9, 12, 15–18). Here, we examined the clonality of those T cell repertoires in tumors, which contain innate lymphocytes with invariant TCRα chains (Vα4, Vα7.2, Vα19 and Vα24). The applied method (RT–PCR SSCP) has the advantage of assessing clonality and clonal dominance beside the examination of TCR expression. After the amplified αCDR3 cDNA sequences are separated by SSCP electrophoresis, hybridization with a Cα-specific probe visualizes the particular Vα T cell repertoire. The presence of invariant TCRα chains can be judged in this whole repertoire and further confirmed in a more restricted T cell repertoire by a subsequent hybridization with a Jα- or invariant αCDR3-specific clonotypic probe. After establishing SSCP clonality assay for the identification of NKT cells in autoimmune lesions, our method has been applied in other pathological studies and data were confirmed by different methodologies (24–27). Particularly, we modified the method to detect invariant Vα7.2-Jα33 T cells, later termed MAIT cells, in autoimmune lesions (12, 19).

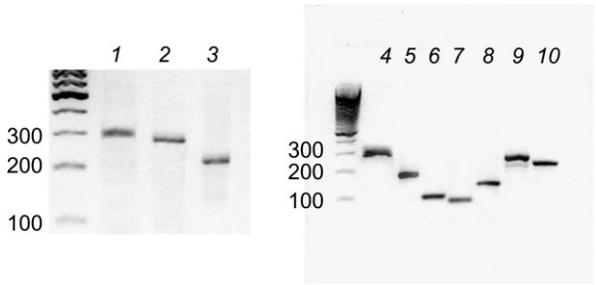

Fig. 6. Expression of Vβ2, Vβ13, MR1 and cytokines in tumor tissues expressing MAIT αTCR. Expression of cytokines, TCR-Vβ chains and MR1 was determined by RT–PCR in a representative sample of clear cell kidney cancer (#1) expressing the invariant MAIT αTCR (see Tables 2 and 3) (lanes indicate: 1 = Vβ13A, 2 = Vβ13B, 3 = Vβ2, 4 = TNF-α, 5 = IFN-γ, 6 = IL-17, 7 = IL-12, 8 = IL-10, 9 = IL-4 and 10 = MR1).

Out of the analyzed four T cell sub-populations, only invariant Vα24-JαQ NKT and Vα7.2-Jα33 MAIT cells could be identified in tumor tissues. Although both Vα4+ and Vα19+ TCR were present in about half of the kidney cancers, no identical clonotypes indicating an invariant αCDR3 were detected in these repertoires. In brain tumors, even the non-canonical Vα4+ and Vα19+ clonotypes were absent. These data suggest that MAIT and NKT cells are the major innate αβTCR lymphocyte subsets to infiltrate human tumors and may indicate that presence of these T cell subsets in tumors is not accidental but rather specific. Indeed, the clonal dominance of MAIT and NKT was superior to PB.

The number of infiltrating Vα7.2 and Vα24 clonotypes varied between two and nine per sample in kidney cancer and there was a great variation in the number and dominancy of certain clonotypes. In contrast, brain tumors had a more restricted number and less dominancy of clonotypes. These data suggested that although Vα7.2+ and Vα24+ T cells were present in both PNS and CNS tumors, kidney cancers were infiltrated with more heterogeneous T cell populations. Alternatively, T cells died in the CNS tumors. We also observed a more restricted Vα7.2+ and Vα24+ T cell repertoire in autoimmune CNS lesions, compared with autoimmune demyelinating lesions of the PNS (12, 21). Accordingly, our data may indicate that within the total Vα24+ and Vα7.2+ repertoire, both MAIT and NKT cells represent relatively more dominant populations in CNS compared with kidney tumors. Moreover, presence of NKT cells in CNS tumors contrasted to autoimmune CNS lesions, where NKT cells were rarely detected despite the presence of conventional Vα24+ T cells (21). Detection of NKT cells in CNS tumors indicates that absence of NKT cells is unique to autoimmune infiltrates and is not related to the special immunoregulation of the CNS.

The presence of MAIT cells in tumors indicated by the expression of invariant MAIT TCR clonotypes is a novel finding, but not unexpected considering the similarities to NKT cells. The anti-tumor response of NKT cells has already initiated human clinical trials to treat cancer (23, 28–30). Presence of NKT cells or the invariant Vα24-JαQ TCR has been shown in

Table 2. Expression of cytokines, MR1 and TCRα chains of T cell populations with invariant CDR3α in clear cell kidney cancers

	1	*2*	*3**	*4*	*5*	*6**	*7*	*8*	*9**	*10*	*11*
TNF-α	+	+	–	+	+	+	+	+	+	+	+
IL-12	+	+	–	+	+	+	+	+	+	+	+
IFN-γ	+	–	+	+	+	+	+	–	–	–	–
IL-17	+	+	–	+	–	+	+	+	–	+	+
IL-4	+	–	–	–	–	–	–	–	–	–	–
IL-5	–	–	–	+	–	–	+	–	–	–	–
IL-10	+	+	–	+	+	–	+	+	–	–	–
MR1	+	+	+	+	+	+	+	+	–	+	+
NKT	+	+	+	+	–	–	+	–	+	+	–
MAIT	+	+	+	+	+	+	–	+	+	–	–

Numbers in italics indicate individual tumor samples. The number of samples corresponds to those in Figures 1 and 2. MAIT and NKT indicate the presence of the invariant Vα7.2-Jα33 and Vα24-JαQ TCR, respectively. The expression of invariant Vα7.2-Jα33 and Vα24-JαQ TCR was examined by RT–PCR SSCP clonotypic analysis, and the expression of cytokines and MR1 was examined by RT–PCR (see Methods). Asterisks indicate those samples where no IL-4, IL-5 and IL-10 mRNA could be detected despite the presence of the invariant MAIT TCRα chain.

Table 3. Expression of cytokines, MR1 and TCRα chains of T cell populations with invariant CDR3α in brain tumors

	*1**	*2**	*3*	*4*	*5*	*6*	*7*	*8**
TNF-α	+	+	+	+	+	+	+	−
IL-12	+	+	+	+	+	+	+	+
IFN-γ	−	−	−	−	−	−	+	+
IL-17	−	−	+	−	−	−	−	−
IL-4	−	−	−	−	−	−	−	−
IL-5	−	−	−	−	−	−	−	−
IL-10	−	−	−	−	+	+	+	−
MR1	−	+	+	+	−	+	+	+
NKT	−	+	−	−	−	+	+	+
MAIT	+	+	−	−	+	+	+	+

Numbers in italics indicate individual tumor samples. The number of samples corresponds to those in Figures 1 and 2. MAIT and NKT indicate the presence of the invariant Vα7.2-Jα33 and Vα24-JαQ TCR, respectively. The expression of invariant Vα7.2-Jα33 and Vα24-JαQ TCR was examined by RT–PCR SSCP clonotypic analysis, and the expression of cytokines and MR1 was examined by RT–PCR (see Methods). Asterisks indicate those samples where no IL-4, IL-5 and IL-10 mRNA could be detected despite the presence of the invariant MAIT TCRα chain.

human tissue samples (21, 31–33), including tumors *in situ* (34–36). However, the expression of the Vα7.2-Jα33 rearrangement was only examined and found in autoimmune and cutaneous sarcoid lesions, suggesting that MAIT cells can infiltrate tissues as well (12, 31). Although the invariant MAIT TCR has not been examined in tumors so far, the restricted Vβ2 and especially Vβ13 TCRs expressed by MAIT cells have been already shown in a number of tumors (37–41). In addition, several studies indicated an *in vitro* cytolytic activity of infiltrating Vβ13+ T cells, particularly with a CD8 phenotype, both characteristic of MAIT cells (39–41). Indeed, kidney and brain tumors expressing the invariant MAIT TCR were infiltrated by CD8+ T cells in our study. These CD8+ T cells also expressed HLA-DR, indicating an activated state. Of note, the invariant MAIT and NKT TCR were co-expressed in about half of the tumor samples regardless of compartmentalization, suggesting that MAIT and NKT cells infiltrate cancers together.

We also examined the presence of CD56 molecule on infiltrating T cells, which can be connected to cytotoxicity and can be expressed by both NKT and MAIT cells (4, 9). However, T cells in kidney cancer and brain tumors were negative for CD56. Therefore, we examined whether peripheral MAIT cells obtained from patients with these cancers alter expression of CD56 compared with healthy controls. Since no antibody is available to detect MAIT cells, we sorted CD56+ and CD56− subsets from the peripheral blood and applied a clonotypic PCR to identify the presence of MAIT cells in these subsets. The invariant Vα7.2-Jα33 TCR message could be amplified in both CD56+ and CD56− subsets, similar to healthy subjects. Thus, although part of peripheral MAIT cells express CD56 in cancer patients, the tumor-infiltrating MAIT cells may comprise a CD56− subset.

MAIT cells represent a novel T cell population with similar phenotypic and functional properties to NKT cells (8, 9, 12, 15–18). Their regulatory role has been already addressed in autoimmunity and may be related to anti-inflammatory cytokines produced or induced by MAIT cells. In mice, they can protect against autoimmune inflammation of the CNS by an increased IL-10 production through interactions with B cells (18). In humans, the invariant MAIT TCR was detected in autoimmune lesions in connection with expression of IL-4 and IL-10 mRNA (12). However, recent data indicated a heterogeneous cytokine production by murine MAIT cells, similar to NKT cells (20, 42). Considering the presence of MAIT cells in tumors, the suggested similarities to human NKT cells with anti-tumor activity due to production of pro-inflammatory cytokines and the functional heterogeneity of murine MAIT cells, we correlated pro- and anti-inflammatory cytokine expression in tumors with the presence of MAIT clonotypes. Pro-inflammatory cytokines were widely expressed in both kidney cancers and brain tumors, as expected. The only abundantly expressed anti-inflammatory cytokine was IL-10, which may both suppress and stimulate anti-tumor immune responses (43). In addition, only pro-inflammatory cytokines were detected in six tumors expressing the invariant MAIT TCR. The correlation of pro-inflammatory cytokines with MAIT clonotypes may indicate that human MAIT cells may have a pro-inflammatory subset, similar to human NKT and murine MAIT cells (20, 42). Besides expression of cytokines, tumors also expressed MR1, the antigen-presenting molecule of MAIT cells. In the majority of tumors, MR1 was co-expressed with the invariant MAIT TCR, indicating that MAIT cells may have the possibility to be locally activated by ligands presented by MR1 and possibly contribute to the cytokine environment and cytotoxicity (15, 16).

Our data also emphasize the immunological differences of tumors located outside and within the CNS. First, there was a difference in the number and dominancy of both Vα24 and Vα7.2 clonotypes between tumors located in different compartments. In addition, while Vβ2 and Vβ13 expression was obvious in all tumors presenting the invariant MAIT clonotypes in kidney cancer, some of the brain tumors did not disclose Vβ2 and Vβ13 TCR, indicating that a subset of MAIT cells may express other β chains in CNS tumors. In kidney cancer, pro-inflammatory cytokines IFN-γ and IL-17 were abundantly present. In contrast, none of the brain tumors expressed IFN-γ and only one tumor was positive for IL-17 mRNA. This bias was characteristic only of T_h1/T_h17 cytokines since TNF-α and IL-12, cytokines important in anti-tumor responses, were equally well represented in kidney and brain tumors. The number of malignant gliomas and meningiomas was not enough to examine differences regarding MAIT, NKT cells and cytokines.

In summary, our data indicate that a novel NKT-like T cell population, MAIT cells infiltrate tumors similar to NKT cells, while other invariant T cell subsets may not be present. The co-expression of MR1 and MAIT TCR in tumors suggests that MAIT cells may be locally activated. The co-expression of pro-inflammatory cytokines and the invariant MAIT TCR in the absence of T_h2 cytokine messages in tumors may suggest functional heterogeneity of human MAIT cells. Our data also imply that MAIT cells in tumors may belong to a CD56− subset and express CD8 and HLA-DR. Considering the importance of NKT cells in anti-tumor responses represented even by human drug trials, the functional similarities between MAIT and NKT cells and the co-expression of the two invariant TCRs in tumors, our data indicate that beside NKT cells, MAIT cells may be also considered in anti-cancer treatment strategies.

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Abbreviations

CNS	central nervous system
DN	double negative
MAIT	mucosal-associated invariant T
mRNA	messenger RNA
MS	multiple sclerosis
NKT	invariant NKT
PB	peripheral blood
PNS	peripheral nervous system
RT	reverse transcription
SSCP	single-strand conformation polymorphism

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