Implication of γδ T cells in the human immune response to cytomegalovirus

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In normal individuals, γδ T cells account for less than 6% of total peripheral T lymphocytes and mainly express T-cell receptor (TCR) Vδ2-Vγ9 chains. We have previously observed a dramatic expansion of γδ T cells in the peripheral blood of renal allograft recipients only when they developed cytomegalovirus (CMV) infection. This increase was long lasting (more than 1 year), was associated with an activation of γδ T cells, and concerned only Vδ1 or Vδ3 T-cell subpopulations. Analysis of γδ TCR junctional diversity revealed that CMV infection in these patients was accompanied by (a) a marked restriction of CDR3 size distribution in Vδ3 and, to a lesser extent, in Vδ1 chains; and (b) a selective expansion of Vδ1 cells bearing recurrent junctional amino acid motifs. These features are highly suggestive of an in vivo antigen-driven selection of γδ T-cell subsets during the course of CMV infection. Furthermore, Vδ1 and Vδ3 T cells from CMV-infected kidney recipients were able to proliferate in vitro in the presence of free CMV or CMV-infected fibroblast lysates but not uninfected or other herpes virus–infected fibroblast lysates. This in vitro expansion was inhibited by anti-γδ TCR mAb's. These findings suggest that a population of γδ T cells might play an important role in the immune response of immunosuppressed patients to CMV infection.

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Introduction

In humans, γδ T lymphocytes account for a small subset of around 0.5–6% of peripheral blood T cells (1–3) but represent a more substantial fraction of lymphoid cells in areas of the body exposed to the external milieu, such as mucosa (4). The majority (more than 70%) of peripheral blood γδ T cells in the adult human uses the same T-cell receptor (TCR) V region pair Vγ9-Vδ2 and expresses the $CD3^+CD4^-CD8^-$ phenotype (5, 6), whereas the predominant population of γδ T cells (more than 70%) in human intestinal epithelia uses the Vδ1 gene segment and expresses the CD8αα homodimer (4).

Peripheral blood γδ T cells have been found to be enhanced in several pathologies. This peripheral expansion involves Vγ9-Vδ2 T cells in infectious diseases such as tuberculosis (7), leprosy (8), malaria (9), toxoplasmosis (10), or Epstein-Barr virus infection (11). Peripheral Vδ1 T-cell amplification is apparently much rarer, as it has only been described in patients infected with HIV (12) or *Onchocerca volvulus* (13) or suffering from Crohn's disease (14). In vitro, Vγ9-Vδ2 T cells have been shown to recognize native nonpeptidic phosphoantigens broadly expressed by mycobacteria and other pathogens (15, 16). In addition, recognition of MHC class I–like molecules by tumoral intraepithelial Vδ1 T-cell clones has been recently documented (17).

Using animal models of bacterial or parasitic infection in combination with αβ or γδ TCR–deficient mice, several studies have revealed an early protective role of γδ T cells in the host immune reaction (18, 19). The implication of γδ T cells in viral immunity was also suggested in a recent study demonstrating that γδ T cells regulate herpes simplex virus (HSV-1) infection by controlling viral replication and spread (20). In addition, several reports have provided strong evidence of an anti-inflammatory role for γδ T cells through homeostatic regulation of αβ T cells (19, 21, 22), but the ligand specificity that underlies these effects remains unknown. Reciprocal control of γδ T cells by αβ T cells has been reported (23), further accentuating the complexity of the interplay between the 2 T-cell subsets.

Human CMV, a β-herpes virus, is a ubiquitous pathogen with primary infection being followed by lifelong persistence of the virus in its human host. CMV infections are asymptomatic in most immunocompetent individuals but are a major cause of morbidity and mortality in immunosuppressed patients, most notably in organ transplant recipients, patients with AIDS, and

infants infected in utero. CMV infection leads to inflammatory reactions such as severe colitis, retinitis, or pneumonitis (24). In addition, strong evidence has been provided that CMV may be involved in the development of allograft glomerulopathy and chronic rejection in organ transplant recipients (25–27), although the mechanisms underlying this association remain elusive.

Recovery from CMV infection has been correlated with the detection of CMV-specific cytotoxic $CD8^+$ T lymphocytes in kidney recipients (28). Phenotypically, CMV infection leads to enhanced percentages and absolute numbers of $CD8^+$ T lymphocytes in the peripheral blood, which persist for several years in allograft recipients (29). Thus, CMV is able to affect T-cell subset distribution strongly in patients in whom immunosuppressive treatment may disturb regulatory homeostatic processes.

We recently noted the marked expansion of γδ T cells in the peripheral blood of renal allograft recipients who developed a CMV infection (30). To gain further insight into the mechanisms responsible for this γδ T-cell expansion during the course of the immune response to CMV, we analyzed the repertoire of γδ T cells that are stimulated in vivo during CMV infection and the in vitro response of these cells to CMV antigens.

Methods

Patients. After giving their informed consent, 65 kidney allograft recipients comprising 60 patients with a first renal transplant were monitored (without any selection criteria) for the year following transplantation. This population was composed of 41 males and 24 females with a mean ± SD age of 41.6 ± 12.1 years (range: 12–64 years). Of these, 29 had an immunosuppressive regimen combining cyclosporin A, azathioprine, and corticosteroids that was started the day of transplantation, with high doses of corticosteroids that were gradually decreased until 3 months after transplantation. The 36 other patients had a sequential quadritherapy composed of a 10-day induction with antithymocyte globulins (ATG; Fresenius, Bad Homburg, Germany) followed by the same 3-drug therapy as just described. Acute rejections were treated with corticosteroid boluses and, in the case of corticoresistance, with ATG.

CMV infection was diagnosed based on CMV pp65 antigen positivity in peripheral blood leukocytes (Argene Biosoft, Varilhes, France) associated or not with consistent symptoms (fever, pneumopathy, leukopenia, hepatic cytolysis). Diagnostic tests were performed according to the manufacturer's instructions. CMV antigen was monitored weekly during the first 3 months after transplantation, then monthly for 9 months. At least 10 cells per 2×10^5 leukocytes had to be stained for the patient to be considered CMV antigen positive.

Antibodies. The mouse mAb's used for flow cytometry analysis were purchased from the following sources. Phycoerythrin-conjugated anti-CD45RA, anti–HLA-DR, and anti-CD69 were from Becton Dickinson Immunocytometry Systems (Mountain View, California, USA). Phycoerythrin-conjugated anti-CD28, anti-CD25, anti-CD8α, and anti-CD45RO; FITC-, phycoerythrin-, or cyanin-5–conjugated anti-αβ TCR; anti-γδ TCR, anti-CD3, and anti-CD45; FITC-conjugated anti-TCR Vδ2 and anti-TCR Vδ3; biotin-conjugated anti-TCR Vδ1; and phycoerythrin- or FITC-conjugated goat anti-mouse IgG or streptavidin were all from Immunotech (Marseille, France). We also used the following unconjugated mAb's specific to human TCR V regions: Vδ1 (R9.12.6.2), Vδ2 (389), Vδ3 (P8.66.1), Vγ2-3-4 (23D12), Vγ4 (94), Vγ5-3 (56.3), Vγ8 (R4.5.1), and Vγ9 (360) (31–34).

Flow cytometry analysis. The phenotypes of γδ T cells were directly determined on whole peripheral blood according to standard immunofluorescence techniques, as described previously (35). γδ TCR expression was analyzed by triple labeling with anti-CD45, anti-CD3, and anti-panδ TCR mAb's. The phenotypes of γδ T cells were determined by double labeling with anti-panδ TCR mAb's and mAb's directed against various activation antigens. TCR Vγ and Vδ use was evaluated by double labeling with anti-Vγ– and/or anti-Vδ–specific mAb's and anti-CD3 mAb. Negative controls for each labeling consisted of incubation with isotype-matched unrelated mAb's. Fluorescence was analyzed on a FACScan (Becton Dickinson Immunocytometry Systems). The total lymphocyte population was gated on the basis of forward-angle and side-scatter analyses. A minimum of 5,000 lymphocytes was counted for percentage determinations. Results are expressed either as percentages of $CD3^+$ T lymphocytes or as absolute numbers of T-cell subsets, determined by multiplying the percentages reported by the flow cytometer by the absolute lymphocyte count given by an automatic hemocytometer (Technicon H2; Bayer, Leverkusen, Germany). For in vitro studies, γδ T-cell percentages and Vδ-chain use in cultured cells were determined by triple labeling with anti-CD3, anti-αβ TCR, and anti-γδ TCR mAb's or by double labeling with specific anti-Vδ and anti-CD3 mAb's.

PCR analysis of Vγ-Cγ and Vδ-Cδ junctional diversity. After isolation by Ficoll density gradient centrifugation (Eurobio, Les Ulis, France), patients' PBMCs (5×10^6) were used to prepare total RNA according to the guanidium thiocyanate-phenol-chloroform method (36). Total RNA was transcribed into cDNA according to the procedures described by the manufacturer (GIBCO BRL, Gaithersburg, Maryland, USA). The distribution of expressed γδ-chain CDR3 lengths was studied with the Immunoscope technique (37, 38). Briefly, cDNA copies from 0.2 mg of RNA were amplified in 50 μL with 1 of the 3 primers specific to TCR Vδ families, in combination with a Cδ or Cγ primer. Forty amplification cycles were performed using the following schedule: 45 seconds at 94°C; 45 seconds at 60°C; 45 seconds at 72°C; and a final polymerization step of 4 minutes at 72°C. Aliquots (2 μL) of the 3 V/C PCR products were copied in 1-cycle runoff reactions (10 μL) with a fluorescent-labeled, Cδ-specific oligonucleotide. Each labeled runoff product was loaded onto 6% acrylamide sequencing gels for size and fluorescence intensity determination on DNA sequencer 373A (Applied Biosystems, Foster City, California, USA) and analyzed by the Immunoscope software (38). The Vδ gene family–specific primers used, oriented 5′ to 3′, were as follows: Vδ1 (CTGTCAACTTCAAGAAAGCAGCGAAATC), Vδ2 (TACCGAGAAAAGGACATCTATGGC), Vδ3 (GGGGATAACA GCAGATCAGAAGGT), Cδ (TGGGAGAGATG CAATAGCAGGATC), and Cδ-fam (ACGGATGGTTTGGTAGA-GGCTGA).

DNA cloning and sequencing of Vδ1-Jδ junctions. Preparation of PBMC RNA, reverse transcription, and PCR amplification were performed as described previously using the 5′ primer located in Vδ1 region and a 3′ primer in Cδ (33, 39). Amplified DNA was cloned using the pGEM-T Easy Vector System (Promega France, Charbonnières, France), and sequencing was carried out on recombinant plasmids purified from bacterial clones.

Preparation of CMV- or HSV-infected fibroblast lysates, and free CMV. Human foreskin fibroblasts, grown in DMEM containing 10% FCS and 2 mM glutamine, were infected with the Ad169 strain of human CMV or the 1471 strain of HSV at a multiplicity of 1 plaque-forming units (PFU) per cell. After virus adsorption for 2 hours at 37°C, cells were washed with PBS and covered with fresh growth medium. Four days after infection for CMV, and 24 hours after infection for HSV, when cytopathic effects were ≥90%, cells were washed with PBS, scraped into PBS, pelleted, and sonicated in PBS on ice for 2 × 10 seconds, with 1 pulsation per second at an amplitude of 70 and wattage of 12 with 1 minute of cooling between each 10-second burst. Sonicates were clarified at 1,000 *g*

for 20 minutes at 4°C. The protein content of supernatants was determined using a kit from Bio-Rad Laboratories Inc. (Hercules, California, USA), and aliquots were frozen at –80°C. Noninfected (NI) cells grown in parallel were mock infected by undergoing a medium change, and antigens were prepared by sonication as already described. All virus stocks and cells were negative for the presence of mycoplasma.

To produce free CMV (Towne strain), MRC-5 cells were infected at an moi of 0.1 and incubated for 10 days at 37°C in culture medium without serum. The supernatant was harvested and was first centrifuged for 15 minutes at 1,200 *g* to eliminate cell debris. The virus was then concentrated by centrifugation at 6,900 *g* for 18 hours. The pellet was resuspended in PBS (1:100) without Ca^{2+} or Mg^{2+} and sonicated 3 times for 2 minutes in an ultrasonic bath. The protein concentration of the virus preparation was adjusted to 1 mg/mL, and the material was stored at –70°C. The preparation had a titer of $10^{4.25}$ PFU/mL. A control was prepared as follows: MRC-5 cells were mock infected and incubated for 10 days, and the supernatant was treated as already described.

Culture of PBMCs from kidney recipients. PBMCs were isolated by centrifugation on a Ficoll density gradient from 20 mL of the patient's peripheral blood. Cells were seeded in 1 mL of RPMI-1640 (Life Technologies, Cergy Pontoise, France) supplemented with 8% FCS and 2 mM glutamine, at 5×10^5 or 10^6 cells per well in 24-well culture plates. To evaluate the γδ T-cell response to CMV antigens, PBMCs were cultured in the presence of 20 μg/mL of CMV-infected, HSV-infected, or NI fibroblast lysates (see earlier discussion). After 4 days of culture, viable cells were counted on a hemocytometer by eosin dye exclusion, and the percentage of total γδ T cells or TCR-Vδ1–, TCR-Vδ2–, or TCR-Vδ3–expressing T cells was determined by flow cytometry. The amplification ratio was calculated as follows: $(N_{CMV} \times A_{CMV})/(N_{NI} \times A_{NI})$, where N represents the total number of cells recovered after culture and A corresponds to the percentage of γδ T cells labeled with a specific mAb. In some experiments, γδ TCR triggering was prevented by adding an anti–pan-δ mAb (510 ascites diluted 1:500 vol/vol) (16). Anti-gp190 (leukemia inhibitory factor receptor) mAb (ascites diluted 1:500) was used as a negative control (40).

Statistical analysis. To quantify the deviation from normality of a T-cell repertoire as measured by combination of Immunoscope and flow cytometry, we introduced an "index of oligoclonality" in 4 steps. In the first step, a normalized repertoire was calculated for each CMV⁻ patient, combining Immunoscope- and flow cytometry–derived data (see Figure 5); for each combination of the repertoire (δ chain, CDR3 size), its contribution to the total δ repertoire of the patient is equal to the area of the corresponding peak in the Immunoscope profile, divided by the sum of all peaks found in the same profile, and multiplied by the percentage of T-cells expressing the corresponding δ chain, as measured by flow cytometry. In the second step, an average repertoire was computed: the average contribution of each combination of the repertoire (δ chain, CDR3 size) to the average repertoire was calculated as the arithmetic mean of the contributions of the same combination to the repertoires of all CMV⁻ patients. Third, an index of oligoclonality was computed for the δ repertoire of each patient. This index is defined in equation 1 as follows:

$$\text{Index of clonality} = \sqrt{\sum_{(\delta,\ CDR3)} \left(cont_{(\delta,\ CDR3)} - \overline{cont}_{(\delta,\ CDR3)}\right)^2}$$

wherein $cont_{(\delta,\ CDR3)}$ and $\overline{cont}_{(\delta,\ CDR3)}$ are the contributions of the $(\delta,\ CDR3)$ combination to the patient repertoire and average repertoire respectively.

Fourth, the logarithm of this index was plotted as a function of the patient, and the statistical significance of the observed distribution between groups of CMV⁻ and CMV⁺ patient was tested with an unpaired *t* test.

Results

CMV infection induced dramatic and long-lasting expansion of circulating γδ T cells in kidney recipients. The influence of CMV infection on γδ T-cell percentages in the peripheral blood of renal transplant recipients was analyzed longitudinally in 65 patients for at least 1 year from the time of transplantation. CMV pp65 antigen positivity in peripheral blood leukocytes and γδ T-cell percentages of total circulating T cells were monitored monthly. Twenty-two of the 65 patients developed typical CMV infections (CMV⁺ patients), whereas the other 43 patients remained free of any sign of CMV infection (CMV⁻ patients). In agreement with previous studies (24), CMV infection occurred shortly (39 ± 19 days) after transplantation, corresponding to the 2-month period when the immunosuppressive regimen is the most intense.

The mean percentage of circulating γδ T cells remained stable and was less than 4% of total T lymphocytes in the group of CMV⁻ patients (Figure 1). In contrast, CMV infection induced a rapid and dramatic rise in the percentage of γδ T-cells in the peripheral blood of CMV⁺ patients. In the latter patients, the mean ± SEM level of γδ T cells reached 13.4 ± 1.7% of T lymphocytes 4 months after transplantation and then plateaued at this level for at least 1 year. Fifteen CMV⁺ patients monitored for more than 3 years after transplantation maintained their maximum γδ T percentage. Similar results were obtained when data were expressed as absolute numbers of γδ T cells per microliter of blood (data not shown), indicating that the percentage of γδ T cells was not augmented because of αβ T-cell loss under immunosuppressive treatment, but rather because of an actual in vivo expansion of this γδ T-cell subset.

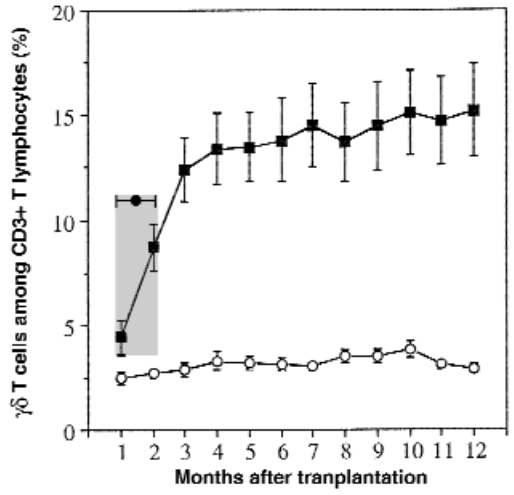


Figure 1
CMV infection induced an expansion of peripheral γδ T cells in kidney recipients. γδ T-cell percentages of peripheral blood leukocytes were determined monthly in 65 patients for 1 year after transplantation. γδ TCR expression was determined by two-color flow cytometry using anti-panδ mAb with anti-CD3 mAb. Results are expressed as mean ± SEM percentages of γδ T cells among total T cells for 43 CMV⁻ patients (open circles) and 22 CMV⁺ patients (filled squares). The mean (filled circle) ± SD (shaded area) onset of CMV infection (39 ± 19 days) is indicated by horizontal bar and shaded area.

γδ T cells from CMV⁺ patients carried several activation markers. To investigate whether the γδ T-cell expansion in CMV⁺ patients was associated with activation of these cells, we used flow cytometry to analyze a number of cell surface markers indicative of an activated phenotype. The majority of γδ T cells from CMV⁻ patients expressed appreciably less HLA-DR, CD69, and CD8α ($P < 0.05$) than did those from CMV⁺ patients (Figure 2). On the other hand, CD25 was equally very weakly expressed by γδ T cells from CMV⁻ and CMV⁺ patients. The most marked differences between CMV⁺ and CMV⁻ patients concerned CD45 isoforms and CD28 cell surface expression. γδ T cells from CMV⁻ patients were CD45RO⁺CD45RA^{low}, a phenotype of memory T cells, whereas those from CMV⁺ patients expressed a CD45RO⁻CD45RA^{high} phenotype ($P < 0.0005$ and $P < 0.05$, respectively). CD28 was virtually absent from the surface of γδ T cells in CMV⁺ patients, whereas at least half of the cells from CMV⁻ patients were positive for this marker ($P < 0.0005$).

Taken together, these data support the idea that once kidney recipients became infected by CMV, their γδ T cells were activated in vivo, thereby suggesting that the expansion of this subset was associated with in vivo antigenic stimulation.

Expansion of γδ T cells concerned only Vδ1 and Vδ3 T cells. The TCR repertoire of γδ T cells in kidney recipients was then analyzed to determine whether the expansion observed in CMV⁺ patients involved all these cells or only a subset. TCR Vδ and Vγ use at the protein level was assessed by flow cytometry on peripheral γδ T cells from 12 CMV⁻ and 28 CMV⁺ patients, using mAb's that specifically recognized each TCR V region. γδ T cells from CMV⁻ patients carried mainly Vδ2 and Vγ9 TCR (Figure 3b), as reported for circulating lymphocytes from healthy donors (5). In contrast, γδ T cells from CMV⁺ patients expressed mainly Vδ1 and, to a lesser extent, Vδ3 TCR (Figure 3a). The mean numbers of Vδ1 and Vδ3 T cells were, respectively, 16.2- and 9.2-fold higher in CMV⁺ patients than in CMV⁻ patients ($P < 0.0001$), whereas the numbers of Vδ2-expressing T cells were comparable in both groups. The Vγ gene-segment use in CMV⁺ patients' γδ T cells was more heterogenous, with all variable regions being represented (Figure 3b). The mean numbers of Vγ2-3, Vγ4, Vγ5-3, and Vγ8 T cells were increased 13.7 ($P < 0.0001$), 18.7 ($P < 0.0001$), 7.0 ($P < 0.005$), and 17.8 ($P < 0.0001$) times, respectively, in CMV⁺ compared with CMV⁻ patients, whereas the Vγ9 cell level was increased only by a factor of 2.5 (not significant).

To determine whether expanded populations preferentially used certain Vδ-Vγ combinations, three-color labeling with anti-CD3, anti-Vδ, and anti-Vγ mAb's was carried out on peripheral T lymphocytes from 5 CMV⁻ and 15 CMV⁺ patients. As expected, Vδ2 expression was always associated with Vγ9 expression in all patients (Figure 4, a and b). When detected in sufficient numbers, Vδ1 cells from CMV⁻ patients showed a slight preference for Vγ2-3 or Vγ5-3 use. The frequency of Vδ3 cells in these patients was generally too low to allow accurate flow cytometry estimation of Vγ region use. In CMV⁺ patients, Vδ1 and Vδ3 cells utilized diverse Vγ regions with a slight predominance of Vγ8-Vδ1 and Vγ9-Vδ3 cells. Extreme situations were observed in 3 of 15 CMV⁺ patients in whom the amplified γδ T-cell population expressed only a single V region combination (Vγ8-Vδ1 in 2 cases and Vγ9-Vδ3 in the case shown in Figure 4c), which represented 15%, 30%, and 27% of peripheral T cells, respectively.

Thus, γδ T-cell expansion after CMV infection involved mainly Vδ1 or Vδ3 T cells but left the pool of Vγ9-Vδ2 T cells that predominated in CMV⁻ patients virtually unaffected.

Junctional diversity of Vδ3 T cells, and to a lesser extent, Vδ1 T cells, was highly restricted in CMV⁺ patients. The selective expansion of T cells bearing particular TCR Vδ subsets during the course of CMV infection suggested that this skewed

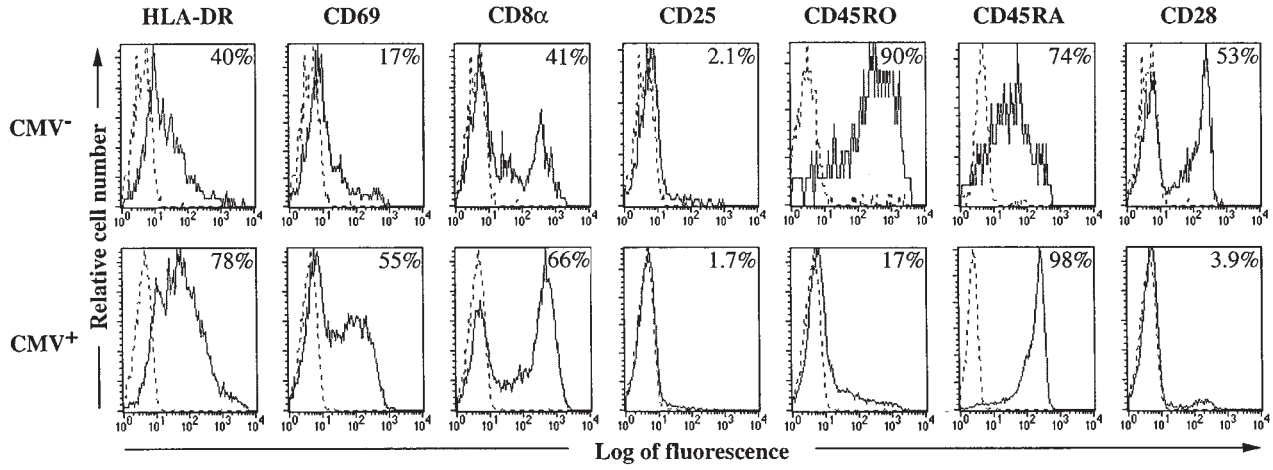


Figure 2

γδ T cells of CMV⁺ patients expressed an activated membrane phenotype. Whole peripheral blood cells from 13 CMV⁻ patients and 17 CMV⁺ patients were labeled with anti-panδ mAb and mAb's directed against the indicated activation markers. The histograms represent the fluorescence intensity of γδ TCR–positive T cells for 1 representative CMV⁺ and CMV⁻ patient. Histograms corresponding to each mAb (solid line) are superimposed with that of the negative control (dashed line) performed with an isotype-matched unrelated mAb. Percentages of positive cells are indicated. The *P* values determined by using Mann-Whitney test between CMV⁺ and CMV⁻ patients were: $P < 0.05$ for HLA-DR, CD69, CD8, and CD45RA; $P < 0.0005$ for CD28 and CD45RO; not significant for CD25.

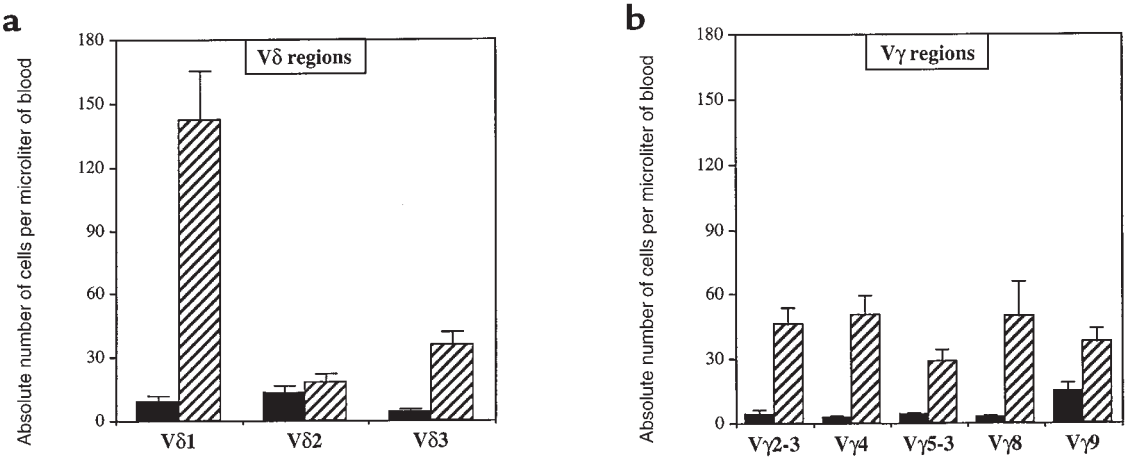


Figure 3

γδ T-cell expansion in CMV⁺ patients involved Vδ1 and Vδ3 T cells. Expression of Vδ (**a**) and Vγ (**b**) chains by circulating γδ T cells from kidney recipients was evaluated by flow cytometry after double labeling with FITC-conjugated anti-CD3 mAb and specific mAb's directed against V regions. Total lymphocyte counts in peripheral blood were determined with an automatic hemocytometer. Results are expressed as mean ± SEM of absolute numbers of positive cells per microliter of blood for each γδ T-cell subset determined in 12 CMV⁻ patients (filled bars) and 28 CMV⁺ patients (hatched bars). Statistical significance between CMV⁻ and CMV⁺ patients for each variable region staining was tested using the Mann-Whitney test: $P < 0.0001$ for Vδ1, Vδ3, Vγ2-3, Vγ4, and Vγ8; $P < 0.005$ for Vγ5-3; not significant for Vδ2 and Vγ9.

repertoire could result from stimulation by a restricted set of antigens. To assess the repertoire diversity of expanded γδ T cells, we studied the CDR3 size distribution of TCR δ chains expressed by patient PBMCs, using the Immunosope method (37, 38). Vδ1-Cδ, Vδ2-Cδ, and Vδ3-Cδ rearrangements were analyzed in 6 CMV⁻ and 11 CMV⁺ patients at least 2 months after transplantation, i.e., when all patients had recovered from the lymphopenia induced by the early phase of immunosuppressive therapy and after the critical period of CMV infection. For CMV⁻ patients, only those with negative CMV serology were analyzed so as to ensure that their γδ T cells were indeed naive with respect to CMV. Vδ1, Vδ3, and to a lesser extent, Vδ2, TCR chains expressed by T cells from CMV⁻ patients showed diverse junctional lengths that were broadly distributed (Figure 5). In contrast, the junctional lengths of Vδ3-Cδ rearrangements in T cells from CMV⁺ patients were much more restricted, and only 1 major CDR3 peak was detected in at least one third of the patients. Note that this (most probably) monoclonal Vδ3 population reached as high as 27% of peripheral T cells in 1 patient. Vδ2 and Vδ1 profiles varied from one CMV⁺ patient to another, but overall the Vδ1 repertoire was more restricted in CMV⁺ than in CMV⁻ patients (note patient Pin, in Figure 5, with 30.4% of a biclonal Vδ1 population). We have introduced an index of clonality to quantify the appearance of oligoclonal expansions within the repertoires displayed in Figure 5 (see Methods). This index measures the "distance" between the repertoire of a given donor and an average repertoire computed as the arithmetic mean of the repertoire of the CMV⁻ donors. When we plotted the logarithm of this index as a function of the donor, we observed a correlation between the index of clonality and the CMV infection status (Figure 6). The statistical significance of this correlation was tested with an unpaired *t* test. The computed *P* value we obtained was less than 0.0001.

To prove that the differences between the CDR3 length distributions attributed to CMV⁺ and CMV⁻ patients were associated with CMV infection, we conducted a longitudinal Immunoscope analysis of several patients before and after CMV infection. Primary CMV infection resulted in a complete remodeling of the Vδ1, Vδ2, and Vδ3 repertoires, which remained stable thereafter (Figure 7a). In contrast, in patients undergoing a CMV reactivation (Figure 7b) or in CMV⁻ patients (Figure 7c), the CDR3 length distribution obtained 2–3 weeks and 10–24 weeks after transplantation was unchanged, although in the course of CMV reactivation an important γδ T-cell expansion was observed. Longer longitudinal studies showed that the γδ T-cell repertoire of CMV⁺ patients was stable over time after CMV infection

Table 1

Identification of junctional residues enriched within Vδ1 sequences derived from CMV⁺ patients

		Phenylalanine			Tryptophan	
Origin	Frequency	Percentage	*P* value	Frequency	Percentage	*P* value
Random	108/545	19.8		66/545	12.1	
Plu CMV⁻	3/17	17.6	NS	2/17	11.8	NS
Plu CMV⁺	7/21	33.3	NS	9/21	42.9	0.0006
Cos CMV⁺	5/10	50.0	0.03	0/10	0	NS
Sel CMV⁺	1/9	11.1	NS	4/9	44.4	0.02

The frequency of phenylalanine and tryptophan between positions P3 and P6 in Vδ1 CDR3, starting from glycin of Vδ1, was estimated within Vδ1 sequences derived from the literature (random, $n = 545$) from patient Plu before (CMV⁻, $n = 17$) and after (CMV⁺, $n = 21$) CMV infection, and from patients Cos (CMV⁺, $n = 10$) and Sel (CMV⁺, $n = 9$) after CMV infection. *P* value determined using Fisher's exact test between patient and random. NS, not significant.

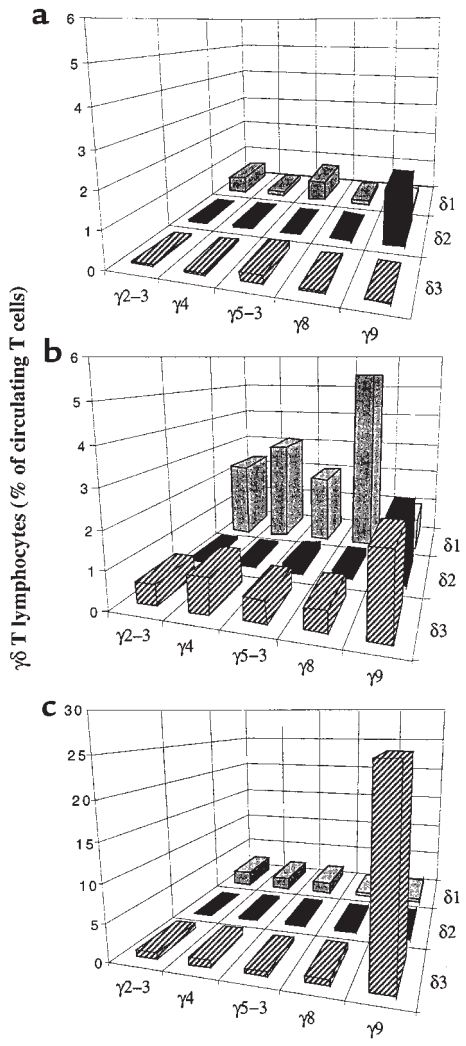


Figure 4
Vδ-Vγ repertoires of γδ T cells from CMV+ and CMV– kidney recipients. Expression of Vδ and Vγ were simultaneously assessed by triple labeling with anti-CD3, anti-Vδ, and anti-Vγ mAb's. Results are expressed as mean percentages of each subset among total peripheral T cells for 5 CMV– patients (**a**), 15 CMV+ patients (**b**), and 1 CMV+ patient (Fer) (**c**) whose expanded cells expressed a single Vδ-Vγ combination.

(Figure 7, a and b), and similar patterns of CDR3 size distribution were obtained from 3 months up to 17 months after transplantation in a given patient (data not shown).

Thus, expansion of Vδ1 and Vδ3 T cells during CMV infection was associated with a restriction of their repertoire, which is suggestive of an antigen-driven selection.

Recurrent junctional motifs in Vδ1+ T cells of CMV+ patients. In an attempt to determine whether expanded Vδ1 T cells (which showed less-restricted junctional size diversity than Vδ3 cells) shared recurrent CDR3-encoded motifs that could be indicative of an antigen-driven selection by structurally related antigens, we further analyzed several Vδ1 junctional sequences derived from the PBMCs of 1 patient before and after CMV infection. As shown in Figure 8, Vδ1 sequences recovered before CMV infection were highly diverse, but a few of them were found 2–4 times. These repeated sequences, which are not uncommon in healthy adults (41), probably reflect previous clonal expansions driven by recurrent peripheral antigens. Consistent with Immunoscope studies, CMV infection resulted in profound changes of the Vδ1 repertoire. Indeed, no single sequence was shared before and after infection, including the repeated ones (which were found up to 7 times after CMV infection). It is noteworthy that these repeated sequences were identical at the protein level but often differed at the nucleotide level (data not shown). Interestingly, although there were no obvious CDR3 length constraints, several residues seemed to be selected or counterselected in a large fraction of sequences derived from expanded Vδ1 cells (Figure 8). In particular, phenylalanine and tryptophan, 2 apolar aromatic bulky residues, were found between positions P3 and P6 on the CDR3 loop of, respectively, 7 of 21 and 9 of 21 distinct Vδ1 sequences after CMV infection. In contrast, sequences carrying phenylalanine and tryptophan at these positions represented, respectively, only 3 of 17 and 2 of 17 sequences derived from the same patient before infection (Figure 8), and at most, 19% and 12% of 545 random Vδ1 sequences derived from the literature (Table 1). Importantly, selection of similar aromatic residues was also observed in 2 other CMV+ patients, whose Vδ1 sequences carried a phenylalanine between P3 and P6 in 5 of 10 cases for patient Cos, and a tryptophan between P3 and P6 in 4 of 9 cases for patient Sel (Table 1).

Taken together, these observations strongly indicate that CMV infection was associated with the selective expansion of Vδ1 T cells bearing specific junctional motifs.

Vδ1 and Vδ3 cells from CMV+ patients responded to CMV antigens in vitro. Because the findings described here suggested that γδ T-cell subsets were selected during CMV infection in vivo, we next investigated whether these cells were able to respond to CMV antigens in vitro. PBMCs isolated from CMV+ kidney recipients were incubated with lysates of fibroblasts previously infected or not with CMV, and γδ T-cell numbers and percentages were determined after 4 days of culture. Higher numbers (Figure 9a) and percentages (Table 2) of γδ T cells were regularly recovered from cultures supplemented with lysates containing CMV antigens (CMV+ lysates) when compared with cultures with lysates of NI fibroblasts (NI lysates). The mean ± SD amplification ratio for the 14 CMV+ patients tested was 3.0 ± 0.8 (Figure 9b). In contrast, the stimulating effect of CMV lysates was less potent on γδ T cells from CMV– patients because the mean amplification ratio for the 8 CMV– patients tested reached only 1.4 ± 0.3 (Figure 9b). These results strongly suggested that prior in vivo activation was required to obtain an adequate in vitro response. The ability of CMV to stimulate γδ T-cell proliferation was also observed when used as free virions enriched by infected fibroblast supernatants (Figure 9c). Moreover, the stimulating effect of enriched free CMV or CMV+ lysates was not lost when they were incubated at 56°C for 30 minutes to inactivate the virus (Figure 9c). The addition of mAb's specific for MHC class I (W6/32), CD1a (OKT6), CD1b (4A7.6), CD1c (L161), and CD1d (51.1) did not block the proliferation of γδ T cells induced by CMV extracts (data not shown). This suggests that recognition of CMV-relat-

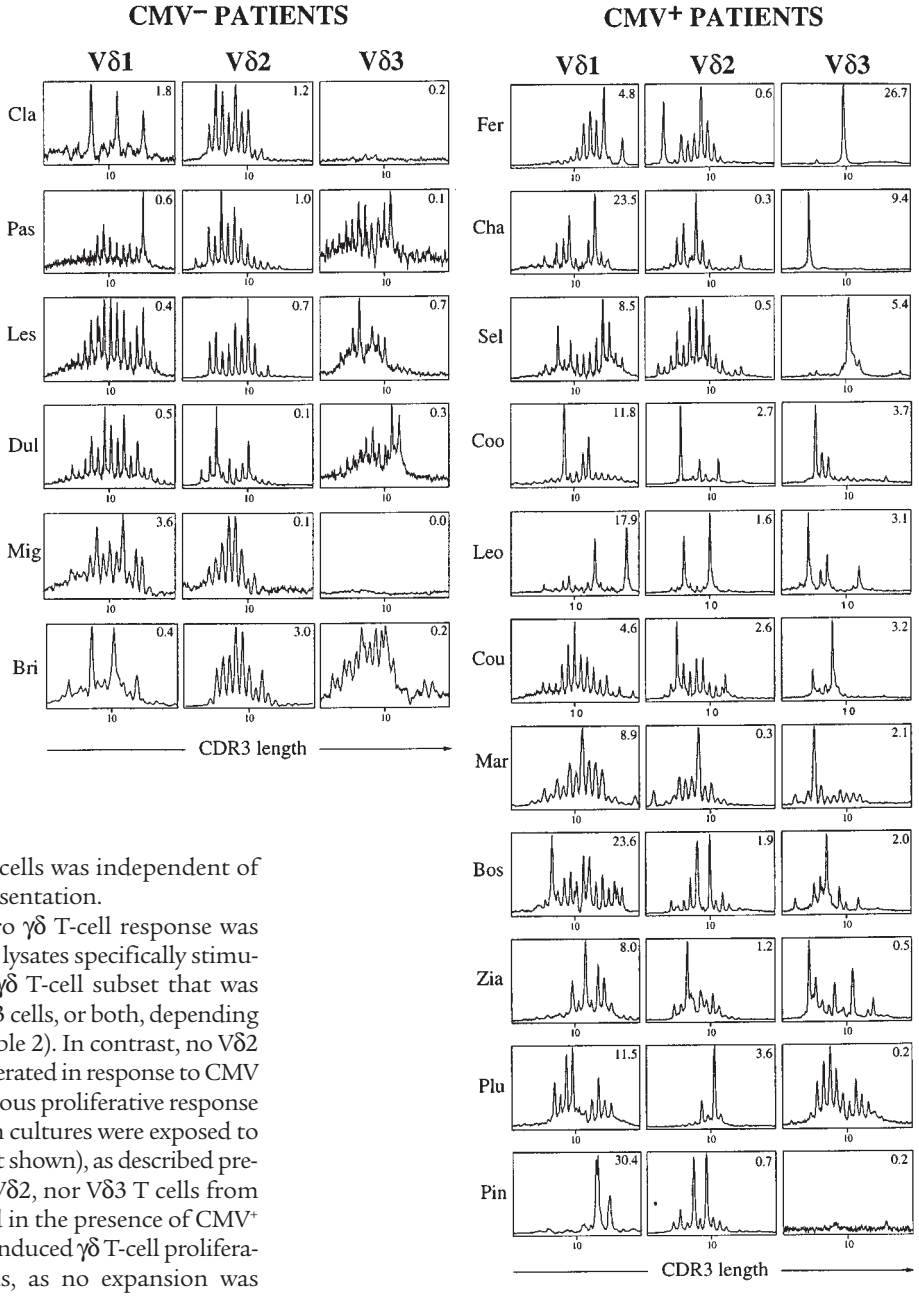


Figure 5
Junctional diversity of Vδ1 and Vδ3 T cells was more restricted in CMV⁺ than in CMV⁻ patients. Profiles of the fluorescent Vδ-Cδ runoff products obtained with PBMCs from 6 CMV⁻ and 11 CMV⁺ patients. Fluorescence intensity is represented in arbitrary units as a function of the size of single-strand DNA fragments. The number in the top right corner of each panel indicates the percentage of the corresponding cell subset among peripheral T cells.

ed antigens by Vδ1 and Vδ3 T cells was independent of class I and class I–like MHC presentation.

The specificity of this in vitro γδ T-cell response was addressed in 2 ways. First, CMV lysates specifically stimulated the proliferation of the γδ T-cell subset that was expanded in vivo, i.e., Vδ1 or Vδ3 cells, or both, depending on the CMV⁺ patient tested (Table 2). In contrast, no Vδ2 cells from any patient ever proliferated in response to CMV lysates (Table 2), whereas a vigorous proliferative response of these cells was observed when cultures were exposed to mycobacterial antigens (data not shown), as described previously (16). Neither Vδ1, nor Vδ2, nor Vδ3 T cells from CMV⁻ patients ever proliferated in the presence of CMV⁺ lysates (Table 2). Second, CMV-induced γδ T-cell proliferation was specific to this virus, as no expansion was observed with lysates of fibroblasts infected with HSV (Figure 9d) or varicella-zoster virus (data not shown), which both belong to the same viral family as CMV (herpesvirus).

To test whether this in vitro CMV-driven expansion involved the γδ TCR, the proliferation assay was performed in the presence or absence of an anti–pan-δ mAb that is known to block TCR-dependent activation of γδ T cells (16). Indeed, it completely abolished the γδ T-cell response to CMV antigens (Figure 9e), but control mAb had no effect (data not shown), suggesting that the CMV-induced γδ T-cell expansion did not result from a nonspecific soluble or membrane-bound stimulus but was dependent on γδ TCR triggering.

In agreement with this CMV-mediated induction of γδ T-cell proliferation, we also observed an increase of CD69 and, to a lesser but reproducible extent, of HLA-DR, surface expression on these cells when they were incubated with CMV⁺-lysates or with free CMV (Figure 10).

Discussion

Transgenic αβ TCR–deficient mice have been used to elucidate the role of γδ T cells specifically in several models of pathological states. These studies revealed that γδ T cells can have a protective (19, 20, 42) or anti-inflammatory (22, 43) role during the course of the immune response. In the present study, we analyzed the behavior of γδ T cells under circumstances in which αβ T-cell functions are compromised by the immunosuppressive therapy given to kidney recipients. We demonstrated that γδ T cells, with particular CDR3 sizes and specific junctional motifs, were selected, activated, and massively expanded during the course of CMV infection in renal kidney recipients and that these cells were activated in the presence of CMV extracts in vitro.

The γδ T-cell percentages of circulating T cells and their absolute numbers rose dramatically in the peripheral

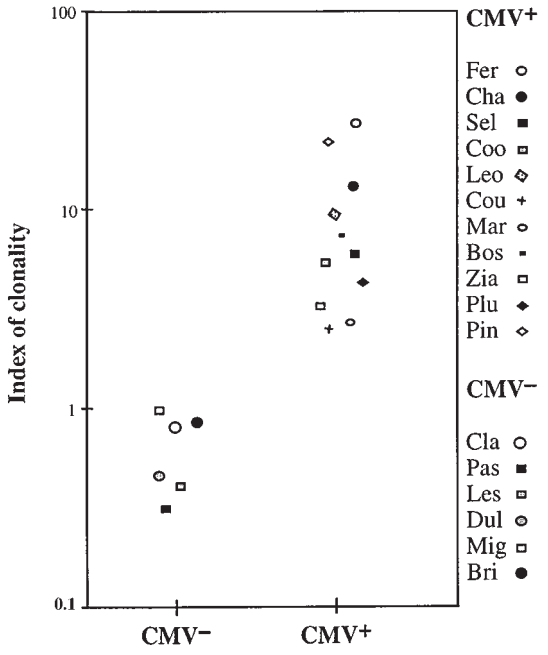


Figure 6
Oligoclonality of Vδ repertoire was more pronounced in CMV+ compared with CMV- patients. Index of clonality for each patient studied in Figure 5 was calculated as indicated in Methods, and the logarithm of this index was plotted as a function of the donor CMV status. $P < 0.0001$ determined with an unpaired *t* test.

blood of transplant patients once they developed a CMV infection. This expansion of γδ T cells was associated with their activation, as they exhibited several markers of an activated phenotype, such as CD69 and HLA-DR. They also expressed surface CD8α, an antigen associated with an activated state in intraepithelial γδ T lymphocytes (4). In addition, γδ T cells from CMV+ patients expressed almost no CD28, a phenotype in agreement with an in vivo oligoclonal expansion of these cells (44–46). As postulated for the Vδ1 T cells of HIV-infected patients (47), the CD45RA+CD45RO- phenotype of γδ T cells from CMV+ patients may indicate that they are in a preactivated state. However, further kinetic studies and separate Vδ1, Vδ2, and Vδ3 T-cell phenotype analysis in kidney recipients indicated that the Vδ1 and Vδ3 T cells predominantly displayed a CD45RO- phenotype. In contrast, Vδ2 T cells expressed the CD45RO marker, either in CMV- or CMV+ patients from the onset of CMV infection (our unpublished observation).

Analysis of Vδ-chain use revealed that peripheral γδ T-cell expansion in the course of CMV infection involved only Vδ1 or Vδ3 T cells. Although Vγ9-Vδ2 cells are prominent among circulating γδ T cells of kidney recipients in the absence of CMV infection and have been shown to recognize a broad spectrum of pathogens (6), the size of this population was not altered by CMV. This apparent absence of Vγ9-Vδ2 T-cell involvement is not due to an anergic status as previously proposed for Vγ9-Vδ2 T cells in HIV+ patients (48), as such cells still proliferated strongly in response to mycobacterial antigens (data not shown) (16). Whereas abnormal expansion of Vδ3 cells has not been reported thus far, that of Vδ1 cells has been observed in several chronic inflammatory diseases (14, 52,53) and occasionally in infectious diseases (12, 13, 49–51). Expansion of Vδ1 cells is generally restricted to anatomical sites affected by the disease and is seldom associated with an increase in peripheral blood. Although the expansion of γδ T cells during CMV infection was observed in blood, the primary site for γδ T-cell activation remains unknown. However, the phenotype of these cells (mainly Vδ1+, Vγ8+, CD8+, and CD28-) is close to that of intestinal intraepithelial γδ T cells (4, 51, 54), and it is noteworthy that gastrointestinal involvement is one of the most common manifestations of CMV infection in allogeneic kidney recipients. Therefore, we postulate that activation and expansion of γδ T cells during the course of CMV infection might primarily occur in the intestinal epithelia, followed by the migration of these activated cells to the peripheral blood.

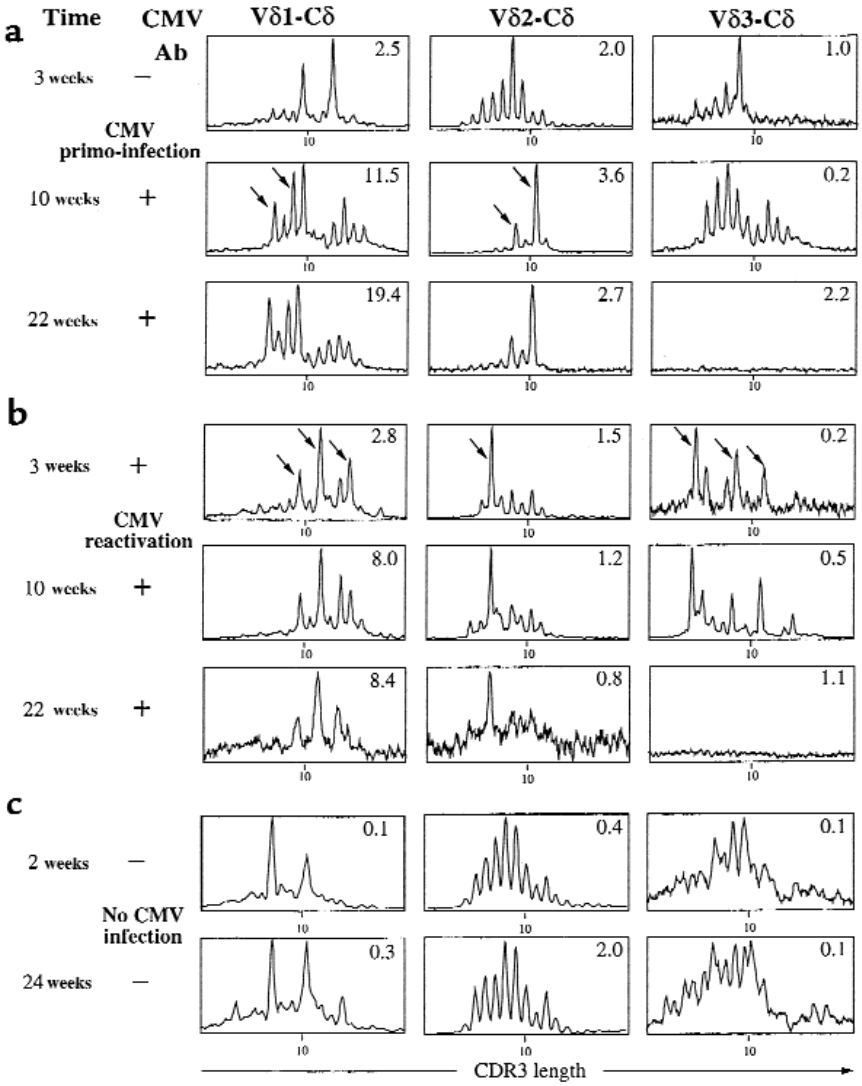
Analysis of the junctional diversity of these amplified γδ

Table 2
Specific amplification of Vδ1 and Vδ3 cells by CMV antigens

	Vδ1		Vδ2		Vδ3	
Patients						
Culture adjunction	Percentage of T cells	Absolute number (× 10^3)	Percentage of T cells	Absolute number (× 10^3)	Percentage of T cells	Absolute number (× 10^3)
Zia (CMV+)						
NI lysates	6.4	39.0	1.0	6.1	1.3	7.9
CMV+ lysates	16.7	147.0	0.7	5.4	1.9	14.6
Fer (CMV+)						
NI lysates	3.7	7.4	0.7	1.4	7.4	14.8
CMV+ lysates	3.9	34.1	0.7	6.1	15.7	137.4
Bre (CMV-)						
NI lysates	5.2	23.7	1.5	6.9	1.4	6.4
CMV+ lysates	6.2	24.8	1.0	4.0	1.3	5.2

PBMCs (5×10^5) freshly isolated from 2 CMV+ patients (Zia, with 8% Vδ1 T cells in peripheral blood; and Fer, with 27% Vδ3 T cells in peripheral blood) and 1 CMV- patient (Bre, with 6% of Vδ1 T cells in peripheral blood) were cultured for 10 days (Zia) or 4 days (Fer and Bre) with NI or CMV-infected fibroblast lysates (20 μg/mL). Viable cells in culture were then counted and phenotyped by labeling with TCR-specific mAb, and absolute numbers of each γδ T-cell subset were calculated. Data shown are representative of at least 20 CMV+ patients and 10 CMV- patients.

Figure 7
Longitudinal study of the γδ T-cell repertoire in the course of CMV primo-infection or reactivation. Shown are the profiles of the fluorescent Vδ-Cδ runoff products obtained with PBMCs from 2 patients undergoing either CMV primo-infection (**a**) or reactivation (**b**). Analyses were performed before (3 weeks after grafting) and after (10 and 22 weeks after grafting) CMV infection. CMV Ab indicates the CMV serological status of the patients before and after CMV infection. Profiles obtained 2 and 24 weeks after transplantation from 1 CMV^- patient are also shown (**c**). The number in the upper right corner of each panel indicates the percentage of the corresponding cell subset within peripheral T cells. Note the appearance of new major peaks (arrows) after CMV primo-infection but not after reactivation or in the absence of CMV infection. The Vδ3-Cδ profiles at 22 weeks are typical of those seen when too few cells are accessible.



T cells suggested an oligoclonal selection of Vδ1 or Vδ3 cells in vivo during CMV infection, which seems to involve 2 different mechanisms. First, CDR3 length distribution was restricted for Vδ3 cells in all CMV^+ patients, as was that of Vδ1 T cells, but the latter occurred in some patients only. This junctional diversity was extremely restricted in some patients whose Vδ1 or Vδ3 T cells contained a single population with only 1 or 2 CDR3 lengths and accounted for one third of peripheral T cells. This finding further emphasizes the strength of the γδ T-cell selection and the subsequent expansion of these cells. Second, in Vδ1 cells with various CDR3 sizes, selective antigenic pressure by structurally related antigens was also suggested by the increased occurrence of specific junctional motifs. When considering individual patients, the γδ T-cell repertoire was profoundly altered after the onset of CMV infection, with an overexpression of particular sequences and selection of bulky apolar aromatic amino acid residues in the CDR3 TCR region of the δ chain. These observations are in agreement with the existence of an in vivo antigen-driven selection of Vδ1 or Vδ3 T cells during the course of CMV infection. Consistent with this notion, γδ T cells from CMV^+ patients were specifically activated in the presence of CMV-infected fibroblast lysates in vitro. TCR involvement in this response is strongly suggested by the restricted TCR features of the responding cells and the blocking activity of anti-γδ TCR mAb. Furthermore, the response of Vδ1 and Vδ3 T cells to free CMV, even after thermal inactivation, indicates that they may directly recognize an intrinsic or an envelope CMV component and do not require the in vitro infection of PBMCs during the culture time.

The present observations are reminiscent of several previous studies describing in vivo or in vitro γδ T-cell responses to other infectious agents. In particular, Vδ1 T-cell expansions have been observed in patients infected with HIV or *Onchocerca volvulus*, and within synovial fluid lymphocytes of patients with Lyme arthritis (13, 52). Although in the latter 2 cases, Vδ1 T cells have been shown to respond in vitro to the causative agent, the mechanisms underlying the in vivo expansion of γδ T cells in patients with HIV remain unclear. However, it seems likely that the γδ T-cell responses to HIV and CMV correspond to unrelated phenomena driven by distinct stimuli. Indeed, in vivo γδ T-cell expansions are much stronger after CMV infection than after HIV infection and involve γδ T-cell subsets with different TCR V regions (i.e., Vδ1 and Vδ3 in the former case, Vδ1 only in the latter case) and different TCR junctional features (no restriction of

Figure 8
Analysis of Vδ1-Jδ junctional sequences before and after CMV infection. Shown are the deduced amino acid junctional sequences (single-letter code) of Vδ1 transcripts derived from the PBMCs of 1 patient before and after CMV infection. Phenylalanine and tryptophan residues are boxed when located between positions P3 and P6, starting from glycin of Vδ1. *n*, number of junctional inserts with identical nucleotide sequence. X, amino acid not determined.

Status	% δ1 cells	*n*	Vδ1	CDR3	Jδ
Before	2.5	1	C A L G	L G G C Y	T D K L I F G K I
CMV infection		1	C A L G	K L R G D Y	T D K L I F G K I
		1	C A L G	L G A X R G G D	T D K L I F G K I
		4	C A L G E	L L [F] R R T G G L	D K L I F G K I
		1	C A L G	D H [W] S G G Y G G	T D K L I F G K I
		1	C A L G	D L P R R G V S P	T D K L I F G K I
		1	C A L G E	[W] S S G W G I R V E Y	K L I F G K I
		1	C A L G	G S R T G X Y L A X A	A Q L F F G K 2
		1	C A L G E	R S P P A G G Y L A	A Q L F F G K 2
		1	C A L G	D P R P S H R H W G I G	D K L I F G K I
		1	C A L G E	L G D P I G Y V R Y	T D K L I F G K I
		1	C A L G	G G I S [F] L L K G I L	T D K L I F G K I
		2	C A L G E	P [F] L I R V I L G G	T D K L I F G K I
		3	C A L G	A P X V R S P R V V V R I	D K L I F G K I
		1	C A L G E	H T H R P X Y W G N P D	T D K L I F G K I
		1	C A L G E	L Y S I L L A V L L G X R R E	T D K L I F G K I
		1	C A L G E	G L P X Q H P E I L G T L T P D P R T H S I P	D K L I F G K I
		23			
After	11.5	1	C A L G E	E L V T Y	T D K L I F G K I
CMV infection		1	C A L G	T Q [W] I P Y	T D K L I F G K I
		2	C A L G	A N V V P D	T D K L I F G K I
		1	C A L G E	L [W] R A D	T D K L I F G K I
		1	C A L G	X R D [W] A P Y	T D K L I F G K I
		1	C A L G	G Y [W] G I L D	T D K L I F G K I
		1	C A L G	D L I S [W] G I R	T D K L I F G K I
		1	C A L G	V S A [F] G G P Y	T D K L I F G K I
		1	C A L G	N L V C [W] G X L	T D K L I F G K I
		1	C A L G E	S I S [W] G I R H	D K L I F G K I
		5	C A L G	L [F] P G G P Q G G	T D K L I F G K I
		1	C A L G	X Y S P X W G R Y	T D K L I F G K I
		1	C A L G	S [W] L [F] L G P Q G G	T D K L I F G K I
		1	C A L G	D H [F] L M Y W G I P V S	D K L I F G K I
		1	C A L G	D P A [F] L A P S D T Y	T D K L I F G K I
		1	C A L G	T [F] L S Y W G I R L P	T D K L I F G K I
		1	C A L G	N S Y L G D Y V L D A	T D K L I F G K I
		7	C A L G E	H Q A Y W G I N L P L Y	T D K L I F G K I
		2	C A L G	P S [W] [F] Y E R F Y W G I G R	T D K L I F G K I
		1	C A L G E	A V M E X L P L F L L G E P	D K L I F G K I
		1	C A L G	I K G L P T S L L G I P R S Y	T D K L I F G K I
		33			

Vδ1 repertoire in HIV-infected patients; ref. 47). In addition, the decreased number and the anergy of Vγ9-Vδ2 T cells were observed only in HIV-infected patients (48).

The persistence of γδ T-cell expansions after primary CMV infection, as was documented for at least 1 year, suggests that CMV induces profound and long-lasting modifications of the γδ T-cell repertoire. This could explain why in patients undergoing secondary CMV infections (i.e., whose γδ T-cell repertoire has been previously shaped by an earlier encounter with CMV), the TCR features of γδ T cells remained unchanged after CMV reactivation, despite the expansion of this subset (Figure 7b). Similar observations have been made for CD8 lymphocytosis caused by CMV infection in renal allograft recipients (29). Although CMV antigen positivity in peripheral leukocytes is transient, lasting for only a few days to a few months, CMV remains in host cells for life. This latent or controlled chronic viral residence may be responsible for sustained activation of γδ T cells. Alternatively, long-term persistence of γδ T cells might also be due to the fact that CMV infection occurs precisely at the time of the most profound immunosuppression preceding T-cell regeneration. Thus, we cannot exclude the possibility that CMV-driven γδ T-cell expansion will lead these cells to occupy selected niches once occupied by αβ T cells. In addition, immunosuppressive treatment, particularly cyclosporin A, has been shown to alter homeostatic processes (55), thereby favoring the maintenance of γδ T cells in the periphery. Such important alterations of T-cell populations after CMV infection in allograft recipients may have profound consequences on their future immune responses against other agents. Consistent with this possibility, superinfections with fungal, protozoan, or bacterial pathogens have been frequently associated with CMV infections.

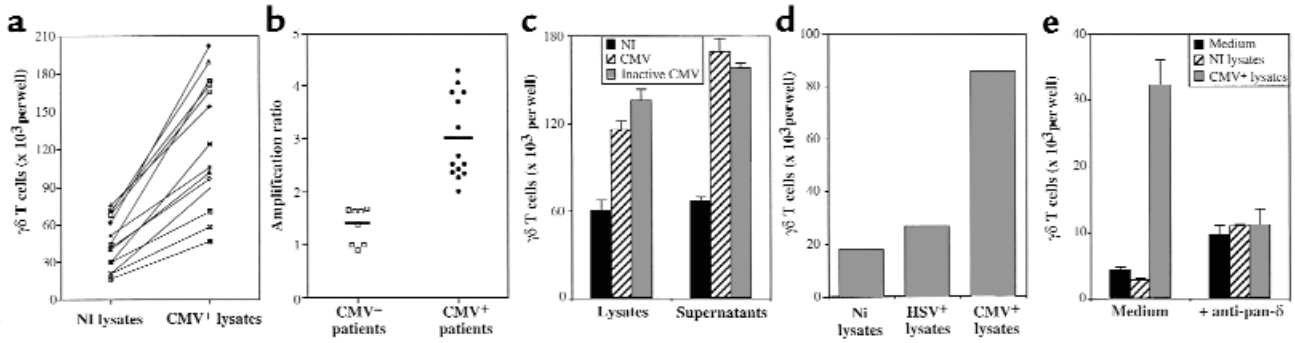
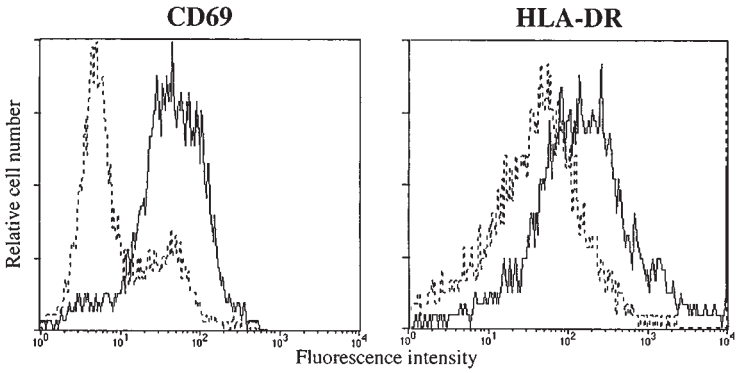


Figure 9

In vitro response of patients' γδ T cells to CMV antigens. (**a**) PBMCs (5×10^5) from each of 14 CMV+ patients were cultured in medium supplemented with 20 μg/mL of either CMV-infected (CMV+) or noninfected (NI) fibroblast lysates. After 4 days, cells were counted and phenotyped with anti-panδ, anti-panβ, and anti-CD3 mAb's. (**b**) Amplification ratios of PBMCs from 8 CMV− and 14 CMV+ patients incubated for 4 days with CMV+ or NI lysates. The mean for each group is represented by a horizontal bar. (**c**) PBMCs (5×10^5) from a CMV+ patient were cultured in the presence of CMV+ lysates (20 μg/mL), free CMV enriched by infected fibroblast supernatant, or the control NI lysates or supernatant. CMV lysates and supernatants were also tested after inactivation by incubation for 30 minutes at 56°C. Results are expressed as means ± SD of culture duplicates and are representative of experiments performed on 5 separate patients. (**d**) PBMCs (5×10^5) from a CMV+ patient were cultured in the presence of 20 μg/mL of NI, CMV+, or HSV+ lysates. Data shown are representative of experiments performed on 5 separate CMV+ patients. (**e**) As in **c**, but anti–pan-δ mAb (1:500 vol/vol) was added to the culture medium to inhibit the γδ T-cell expansion induced by CMV antigens. Results are expressed as means ± SD of culture duplicates and are representative of experiments performed on 7 separate CMV+ patients.

A major unknown that remains is the role played by γδ T cells in immune responses to CMV. Particularly pertinent is the demonstration that γδ T cells protect mice from lethal encephalitis induced by HSV infection, a virus of the same family as CMV (20). Like CMV, HSV can inhibit MHC class I expression by infected cells to avoid recognition by αβ T cells (56–62). In addition, 1 CMV gene (UL18) encodes a β_2-microglobulin–binding protein with homology to MHC class I heavy chains (63). This molecule is a ligand for a newly described natural killer (NK) cell inhibitory receptor (LIR) (64) and was first shown to inhibit NK cell cytotoxic activity (65,66) and then, by contrast, to enhance killing of target cells (67). Thus, CMV evolved mechanisms to evade immune surveillance by MHC-restricted αβ T cells and to affect the activity of LIR-expressing NK cells. However, γδ T cells usually recognize antigen directly in an MHC-independent manner (17, 68, 69), and, accordingly, in vitro CMV-stimulated proliferation of Vδ1 and Vδ3 T cells was not blocked by anti–MHC class I or anti-CD1 mAb's (data not shown). Therefore, γδ T-cell function should not be disrupted either by reduced MHC class I expression or by the UL18 gene product, and it may represent a third line of resistance to CMV. Furthermore, development of γδ T cells in the intestinal epithelium, unlike that of αβ T cells, has been shown to be resistant to cyclosporin A (70). Thus, γδ T cells might be more efficient than their αβ counterparts in providing adequate control of CMV infection in the context of induced immunosuppression in the transplanted patient.

In conclusion, our study constitutes the first evidence for a putative role of γδ T cells in the immune response to CMV and for an oligoclonal expansion of human peripheral Vδ1 and Vδ3 T cells. In addition, CMV infection appears to be a fertile model for the study of these functionally ill-defined subsets of γδ T cells.



	CD69		HLA-DR	
	MFI	Positive cells %	MFI	Positive cells %
NI -----	25.7	28.3	149.7	70.6
CMV ——	81.6	86.0	626.7	92.2

Figure 10

CMV lysates enhanced CD69 and HLA-DR expression on γδ T cells in vitro. PBMCs (5×10^5) from 1 CMV+ patient with 18% of γδ T cells were cultured for 3 days with NI or CMV+ lysates. Cells were phenotyped with anti–pan-δ, anti-CD3 and anti-CD69, or anti-HLA-DR mAb's. Histograms represent the fluorescence intensity of CD3+ pan-δ+ T cells after incubation with NI (dashed line) or CMV+ (solid line) lysates. Data shown are representative of experiments performed on 6 separate CMV+ patients. MFI, mean fluorescence intensity.

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