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CD8 lymphocytosis in primary cytomegalovirus (CMV) infection of allograft recipients: expansion of an uncommon $CD8^+$ $CD57^-$ subset and its progressive replacement by $CD8^+CD57^+$ T cells

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SUMMARY

Allograft recipients undergoing cytomegalovirus infection present increased proportions of circulating $CD8^+$ lymphocytes. A longitudinal study of 11 kidney and five liver allograft recipients with primary CMV infection but no other etiological factor of graft dysfunction revealed selective imbalances of peripheral blood $CD8^+$ T cell subsets. Initially, CMV viraemia is associated with elevated $CD8^{+bright}$ T cell numbers and T cell activation. Activation markers fall to normal when viral cultures become negative (before the end of the first month). During the second to sixth month, most (12/16) patients keep up high $CD8^+$ T cell counts (1050–2900 $CD8^+$ cells/mm^3), comprising an uncommon $CD8^+$ T cell subset, as 45–73% of $CD8^{+bright}$ lymphocytes were $CD3^+$ and $TCR\alpha\beta^+$, but were not stained by anti-CD28, CD11b, CD16, CD56, and CD57 antibody. Unexpectedly, $CD8^+CD57^+$ T cells, a hallmark of CMV infection, do not appear until the second to sixth month of primary CMV infection, and their numbers increase progressively thereafter. They become the predominant $CD8^+$ T cell subset after 6 months of infection and their persistence for several (up to 4) years is strongly correlated ($r = 0{\cdot}87$) with expansion of $CD8^+$ cells. By analysis with MoAbs, there was no bias towards the use of particular TCR-Vβ gene families at any time of primary CMV infection. Persistence of CD8 lymphocytosis is thus directly related to the rate of expansion of an uncommon $CD8^+CD57^-$ subset and its progressive replacement by $CD8^+CD57^+$ T cells that are chronically elicited by CMV.

Keywords T lymphocyte subsets $CD8^+$ cells $CD57^+$ cells cytomegalovirus transplantation

INTRODUCTION

The CD4/CD8 lymphocyte ratio and the pool sizes of the peripheral T lymphocyte compartment appear to be regulated by homeostatic mechanisms [1] that may be altered by some viral infections. $CD4^+$ and $CD8^+$ T cells can expand independently, although $CD8^+$ lymphocytes have a lower expansion potential than $CD4^+$ T cells [1]. Human CMV, a ubiquitous pathogen that is carried lifelong after initial exposure, has profound effects on the CD8 lymphocytic compartment that are increased in immunocompromised hosts, such as solid organ and bone marrow transplant recipients.

Perhaps the most physiologically relevant response to a viral infection is found within the $CD8^+$ T cell population, since cytotoxic $CD8^+$ cells have a major role in the immunological control of viral spread. Accordingly, non-specific natural killer (NK) cell activity and CMV-specific cytotoxic $CD8^+$ T lymphocyte activity have been correlated with the capacity of the host to limit the severity of CMV infection [2,3].

A most remarkable phenotypic feature associated with CMV infection is a consistent increase in the proportion and absolute number of $CD8^+$ T lymphocytes, although CMV-specific T cells are detected in peripheral blood at a frequency of only 1/5000 or lower [2,4,5]. Whereas infection of T cells by CMV is usually abortive [6,7], CD8 lymphocytosis persists for several months in immunocompetent subjects, and even longer in allograft recipients [8,9].

The $CD8^+$ T cell population consists of functionally distinct subsets that can be identified by their differential expression of a variety of cell membrane antigens. Largely reciprocal $CD8^+$ T cell subpopulations express either the CD28 or the CD11b antigen, and differ in their cytotoxic potency [10]. Lymphocytes may also express or lose molecules that characterize minor subsets expanded in some pathologic situations. Much attention has been paid to the CD57 antigen: healthy CMV^+ individuals

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have higher levels of the minor $CD8^+CD57^+$ T cell subset than CMV^- controls [11,12], but CMV-infected allograft recipients maintain a marked expansion of their $CD8^+CD57^+$ lymphocytes [13,14].

However, among the markers that could be considered in CMV infection, the characterization of $CD8^+$ cell surface phenotype in CMV-infected individuals remains limited. To investigate in more detail how selective imbalances of peripheral blood $CD8^+$ T lymphocyte subsets evolve in CMV infection, we performed longitudinal analyses of the subset composition within $CD8^+$ T lymphocytes, comparing allograft (kidney or liver) recipients who become primarily infected by CMV. Our follow-up data show that persisting $CD8^+$ T cell expansion in CMV infection is accounted for by the successive expansion of two minor (in normal subjects) $CD8^+$ subsets; failure to expand these subsets results in transient CD8 lymphocytosis after primary CMV infection.

PATIENTS AND METHODS

Patients

All the patients attending our Transplantation Unit transplanted between February 1990 and July 1993 were enrolled in an immune monitoring programme. All kidney allograft recipients received sequential quadruple drug immunosuppression with anti-lymphocyte globulin (during the first 7 days in most cases), azathioprine and prednisolone. Cyclosporin A was given orally only, and was started when the renal function was stable, usually on the seventh post-operative day. Azathioprine was discontinued when cyclosporin was introduced. The baseline immunosuppressive regimen of liver allograft recipients consisted of triple therapy with prednisolone, azathioprine, and cyclosporin A given intravenously on the first post-operative day and then orally. Acute kidney or liver rejection episodes were treated initially with three courses of steroid pulses, but when ineffective, a mouse MoAb (OKT3) was used as rescue therapy.

Among the patients who were seronegative before engraftment but received an organ from a CMV^+ donor and developed mild (fever, leukopenia) to severe CMV disease, 16 (11 kidney and five liver transplants) were selected because no other etiological factor of graft dysfunction was identified after the diagnosis of CMV infection. These patients had received a prophylactic treatment by anti-CMV-specific immunoglobulin, and only severe CMV disease was treated in three of the liver recipients by a 15-day course of ganciclovir (DHPG).

Virological diagnostic methods

Cultures for CMV were inoculated to MRC5 cells and examined after 24 h and 48 h for detection of immediate–early antigens with the MoAb E13 (Clonatec, Paris, France). Anti-CMV IgM and IgG antibody titres were measured by standard ELISA techniques (Behring, Rueil-Malmaison, France).

Definition of primary CMV infection

CMV infection was defined by viraemia. Viral cultures could indeed be positive before detection of an anti-CMV IgM titre $>1:40$ in formerly seronegative patients. Antigenaemia was obtained since 1992.

Analysis of lymphocyte subsets

EDTA-anticoagulated blood was collected between 7 and 8 a.m. pretransplant, twice weekly from day 1 to 60 post-transplantation, weekly during the third month, and monthly thereafter. The frequency of immunological analysis was increased after CMV infection was diagnosed.

A panel of murine MoAbs was used at concentrations recommended by the manufacturer. All antibodies were directly conjugated with FITC or PE for use on whole blood samples, since Ficoll–Hypaque isolation of mononuclear cells decreases the $CD8^+$ population [15]. Anti-CD4 (T4), anti-CD8 (T8), anti-CD45RA (2H4) and anti-CD56 (NKH-1) antibodies were used at 5 μl/test and purchased from Coulter (Hialeah, FL); anti-CD3 (Leu-4), anti-TCR$\alpha\beta$ (TCR-1,α/β), anti-CD45RO (Leu-45RO), anti-CD28 (Leu-28), anti-CD11b (Leu-15), anti-CD57 (Leu-7), anti-CD16 (Leu-11a), and anti-HLA-DR antibodies were used at 20 μl/test and purchased from Becton Dickinson (Mountain View, CA). Usage of T cell receptor Vβ genes was investigated by a panel of MoAbs against Vβ_2, Vβ_3, Vβ_6, Vβ_8, Vβ_{13}, Vβ_{17} and Vβ_{19} from Immunotech (Marseilles, France). Irrelevant mouse IgG1 (MsIgG1, Coulter) was used as a negative control.

Cells were incubated with the appropriate MoAb, alone or in combination, for 30 min at 4°C and washed with cold PBS. After lysis of erythrocytes (Immunolyse; Coulter), fixation (paraformaldehyde fixative, Coulter), and washing, cells were analysed by flow cytometry using an Epics Profile (Coulter). Fluorospheres (Coulter) were used to calibrate the instrument each day to avoid any shift in fluorescence detection.

Data were collected from at least 5000 cells gated on live lymphocytes by their forward and side scatter characteristics. The absolute number of cells expressing the appropriate marker(s) was calculated by multiplying the percentage of positively stained cells by the blood lymphocyte count.

Results are expressed either as an absolute cell count or as a percentage of the total $CD8^+$ lymphocyte population.

Measurement of soluble IL-2 receptor and of soluble CD4 and CD8 antigen level

Serum concentrations of soluble IL-2 receptor (sIL-2R) and of soluble CD4 and CD8 antigen (sCD8) were measured by standard ELISA techniques (Immunotech, and T Cell Science, Cambridge, MA, respectively).

Statistical analysis

Data are given as medians and ranges. Results were compared by the distribution-free variance method according to Kruskal and Wallis. $P<0{\cdot}05$ was considered statistically significant. Regression curves were calculated according to the linear or logarithmic model.

RESULTS

Primary CMV infection occurred within 3 months after the transplantation of a kidney or a liver from a CMV^+ donor to a CMV^- recipient. Only patients with no other etiological factor of graft dysfunction occurring after the first CMV viraemia were selected and compared with transplant patients with no virological changes.

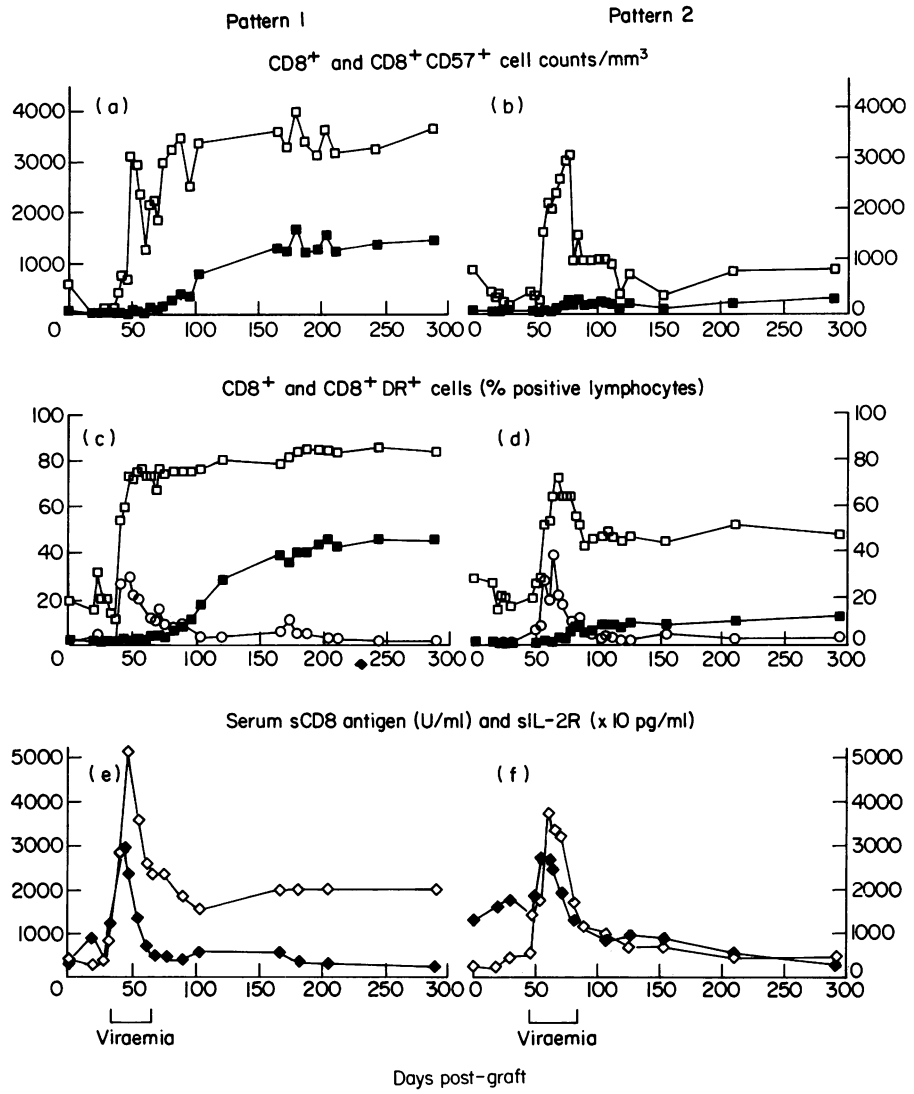

Fig. 1. Two evolutive patterns of $CD8^+$ T cell expansion in primary CMV infection of allograft recipients: pattern no. 1 (a representative patient, left part of the figure) with persistent CD8 expansion, and pattern no. 2 (a representative patient, right part of the figure) with transient elevation of $CD8^+$ cell counts during viraemia. (a, b) Evolution of total $CD8^{+bright}$ T cell counts (□) and of $CD8^+CD57^+$ cell counts (■). (c, d) Evolution of the percentages of $CD8^{+bright}$ T cells (□), of $CD8^+CD57^+$ cells (■) and of $CD8^+HLA\text{-}DR^+$ cells (○). (e, f). Evolution of serum levels of soluble CD8 antigen (sCD8) (◆) and of soluble IL-2 receptor (sIL-2R, levels × 10) (◇).

CD8 lymphocytosis may be transitory or persistent after primary CMV infection

Whereas CD8 lymphocyte numbers were stable at normal values (15–30% of total lymphocytes) in allograft recipients with no virological changes, the most consistent early finding associated with primary CMV infection was an increase of the absolute $CD8^+$ lymphocyte counts. Their rate of expansion was rapid, to peak in less than 1 week, preceding by a few days or accompanying clinical disease and the first viraemia. Only one patient with severe CMV disease had no elevation of his CD8 counts. Concurrent $CD4^+$ T cells were diminished in percentages, but normal or slightly decreased in absolute numbers.

After the viraemic phase (beyond 1 month of infection), two evolutive patterns of CD8 lymphocyte numbers were observed: in most patients ($n = 12$; a representative is shown in Fig. 1a), the absolute number of $CD8^+$ cells remained steadily elevated (1050–2900/mm^3; 42–85% of the total blood lymphocytes) for up to 4 years in our earliest patients. In a second evolutive pattern ($n = 4$), the initial peak of $CD8^+$ cell counts was as high, but the percentage and absolute number of $CD8^+$ cells fell significantly (Fig. 1b). There was, however, no apparent difference in the severity of CMV disease and the viral parameters, the occurrence of rejection before CMV infection, the type of graft (kidney or liver), nor in the immunosuppressive therapy or prescription of ganciclovir treatment between patients differing by persisting *versus* falling CD8 counts after 1 month of infection.

Although $CD8^+$ peripheral mononuclear cells are largely T lymphocytes, they contain some NK cells. Most of the expanded CD8 lymphocyte population (87–97%) expressed brightly the CD8 marker at any time of CMV infection. These cells had the physical properties (forward and right angle scattering) of small

Table 1. $CD8^+$ T cells and their subsets during the successive phases of primary CMV infection in transplant patients with persistent CD8 expansion

	Per cent CD8 in total lymphocytes	Per cent $CD8^+CD28^+$ in $CD8^+$ cells	Per cent $CD8^+CD57^+$ in $CD8^+$ cells	Percent residual $CD8^+$ lymphocytes*
Viraemia	56 (48–73)	61 (44–71) 33 (27–38)†	5 (2–10) 4 (2–7)†	34 (19–54) 64 (58–70)†
Second to sixth month	57 (42–74)	24 (8–38)	19 (5–45)	55 (45–73)
At 6 months	57 (44–85)	26 (12–30)	38 (19–58)	35 (25–48)
At 2 years	62 (50–70)	19 (12–27)	55 (49–74)	26 (14–29)
Seronegatives‡	21 (15–30)	73 (48–87)	10 (5–15)	3 (0–7)

* Residual $CD8^{+bright}$ T lymphocytes that are not stained by anti-CD28, CD11b, CD56, CD57 antibody.

† The data represent two subgroups of patients with and without an initial peak of $CD8^+CD28^+$ T lymphocytes. There were no differences between these two subgroups after the first month of infection, so that the data for later periods of infection were pooled.

‡ Transplant patients who remained CMV^- had stable CD8 counts, and the proportions of their lymphocyte subsets were stable throughout the study period.

Data are given as medians and ranges.

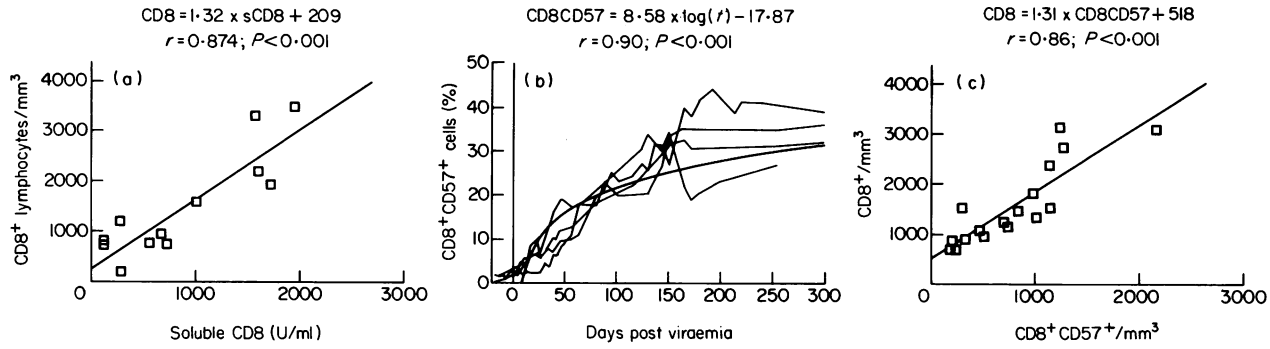

Fig. 2. Persistence of CD8 lymphocytosis in primary CMV infection of allograft recipients is related to the progressive expansion of $CD8^+CD57^+$ T cells and, after 6 months on average, is correlated to $CD8^+CD57^+$ counts and serum levels of soluble CD8 antigen. (a) Regression analysis of $CD8^+$ lymphocyte counts *versus* soluble CD8 antigen (U/ml). (b) Regression analysis of the percentage of $CD8^+CD57^+$ cells *versus* time after the first positive CMV culture. Individual values representative of some patients (——) are represented for comparison with the fitted regression curve (——). (c) Regression analysis of $CD8^+$ lymphocyte counts *versus* $CD8^+CD57^+$ cell counts.

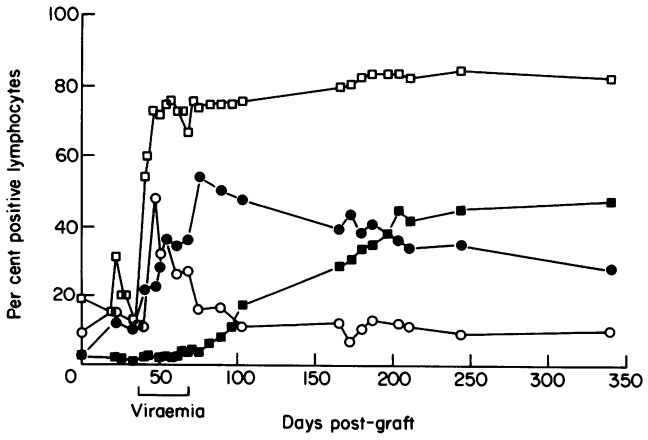

Fig. 3. Evolution of $CD8^+$ T cell subsets in primary CMV infection of allograft recipients with persistent CD8 expansion. A representative patient with an initial elevation of $CD8^+CD28^+$ T cells (○) during the viraemic phase. The $CD8^+CD57^+$ T cells (■) rose afterwards, and replaced progressively an uncommon subset of $CD8^{+bright}$ $CD3^+$ $TCR\alpha\beta^+$ but $CD28^-$ $CD11b^-$ $CD56^-$ $CD57^-$ lymphocytes (●) within the whole $CD8^+$ T cell population (□). Data as percentages of total lymphocytes.

lymphocytes. In two-colour fluorescence, they were also stained by anti-CD3 and anti-$\alpha\beta$ T cell receptor (TCR) antibody, but not by anti-CD16, indicating that the $CD8^{+bright}$ cells are not accounted for by contamination of NK cells, and represent therefore $CD8^+$ T lymphocytes.

Initially in all patients, a significant proportion (34–66%) of $CD8^+$ T cells co-expressed brightly the HLA-DR molecule, and the rate of appearance of $CD8^+$HLA-DR^+ cells was parallel to the increase of the whole CD8 population (Fig. 1c,d). At the viraemic phase, sIL-2R and sCD8 antigen levels also increased markedly in serum, to peak at around six-fold the basal value for sCD8 (Fig. 1e,f), but sCD4 levels did not vary significantly.

Two to 3 months after the first positive CMV culture, the membrane density of the HLA-DR molecule, as indicated by the mean channel of fluorescence, and the number of $CD8^+$HLA-DR^+ lymphocytes, fell to normal values in all patients (Fig. 1c,d). Soluble IL-2 receptor levels also reverted to normal, and sCD8 antigen levels decreased significantly. After the viraemic phase, sCD8 antigen level was thus directly correlated to the size of CD8 lymphocytosis ($r = 0{\cdot}88$; $P < 0{\cdot}001$; Fig. 2a).

Delayed and progressive expansion of $CD8^+CD57^+$ T cells

In all patients during the viraemic period (first month of infection), only 2–10% of $CD8^+$ T lymphocytes co-expressed

the CD57 marker, whether or not elevated CD8 counts were maintained thereafter, and $CD8^+CD57^+$ cell counts were not significantly different from transplant patients with no virological changes (Table 1).

In patients who keep up elevated $CD8^+$ lymphocyte counts, $CD8^+CD57^+$ cells appeared progressively during the second to sixth month after the onset of primary infection. Our longitudinal study of absolute $CD8^+CD57^+$ T cell counts was significant for a delayed and progressive (logarithmic) increase with time, half-maximum values being observed 52 days on average after the first viraemia (Fig. 2b). They became the predominant $CD8^+$ T cell subset (49–74% of $CD8^+$ cells) after the sixth month only (Fig. 1). Then, CD8 lymphocytosis reflected the expansion of $CD8^+CD57^+$ cells ($r=0{\cdot}87$; $P<0{\cdot}001$; Fig. 2c).

Initial CD8 expansion reflects the emergence of an unusual $CD8^+CD57^-$ T cell subset that is replaced progressively by $CD8^+CD57^+$ lymphocytes

At the viraemic period, some patients had an early and transient peak of $CD8^+CD56^+$ and/or $CD8^+CD11b^+$ cells (a maximum of 30% of $CD8^+$ T cells for five patients). In around half of the patients ($n=7$), the predominant lymphocytic population was initially T cells with a $CD8^+CD28^+$ phenotype (44–73% of $CD8^+$ T lymphocytes) (Fig. 3). While in lower absolute numbers, this is also the predominant subset throughout the evolution of allografts with no virological changes (48–87% of $CD8^+$ cells). In the second half of the patients ($n=9$), $CD8^+CD28^+$ T cells were persistently low in percentage at the time of virological diagnosis (Table 1). There was, however, no relationship between the nature of the predominant CD8 subset at the viraemic phase and the maintenance of elevated $CD8^+$ cell counts thereafter.

After the first month of primary CMV infection, for all CMV patients, the number of $CD8^+CD28^+$ T lymphocytes was normal in absolute numbers. It was diminished in percentage (8–38% of $CD8^+$ T lymphocytes) for patients who kept up expanded CD8 values, and in these patients 45–73% of $CD8^+$ T cells did not co-express any of the following subset-specific markers: CD28, CD11b, CD16, CD56, and CD57 (Fig. 3). Their percentages of co-expression of either the CD45RA or CD45RO isoforms did not differ from those of age-matched allograft recipients without virological diagnosis of CMV infection or reactivation (data not shown).

This unusual $CD8^+$ T cell subset persisted beyond the sixth month of primary CMV infection, but their percentages were lower since the $CD8^+CD57^+$ cells became predominant (Table 1).

Analyses of the distribution of T cell receptor Vβ proteins on $CD8^+$ subsets

No significant change in the percentages of $CD8^+$ subsets also stained by MoAbs to $V\beta_2$, $V\beta_3$, $V\beta_6$, $V\beta_8$, $V\beta_{13}$, $V\beta_{17}$, and $V\beta_{19}$ proteins could be detected by longitudinal analysis.

DISCUSSION

Our phenotypic analyses reveal that distinct $CD8^+$ T lymphocyte subsets expand as a function of time in primary CMV infection, allowing one to distinguish three phases in the evolution of CD8 lymphocytosis: (i) the first month, where viraemia is associated with the emergence of activated $CD8^+$ T cells; (ii) during the second to sixth month, in patients who maintain a CD8 lymphocytosis, $CD8^+CD57^-$ T lymphocytes without activation markers predominate and $CD8^+CD57^+$ T cells began to emerge; (iii) $CD8^+CD57^+$ T cells predominate thereafter and persist for several years (up to 4 years in this series).

In all primary CMV-infected graft recipients, expansion of $CD8^+$ T lymphocytes during the viraemic phase (first month of infection) was accompanied by initial co-expression of activation markers (HLA-DR molecule) on most of these cells, as also shown in other studies [16,17], and in the serum sIL-2R and sCD8 antigen levels rose at their maximum values. Both activated and quiescent $CD8^+$ lymphocytes spontaneously release an immunoreactive sCD8 glycoprotein, that has proved useful for monitoring the CD8 cellular compartment in pathologic states [18,19]. Elevation in the serum concentration of sCD8 represents a sensitive marker of $CD8^+$ T cell activation, but also occurs when the pool size of $CD8^+$ lymphocytes increases, both features which characterize the initial phase of primary CMV infection.

After the first month of infection, CD8 lymphocyte counts and their expression of activation markers evolved in the opposite direction in most patients: CD8 lymphocytosis remained steadily elevated, although viral cultures became negative and activation markers disappeared. Only the sCD8 antigen levels persisted over the basal values, and were strongly correlated to $CD8^+$ T cell counts, reflecting the expansion of the CD8 compartment.

However, in contrast with classical reports, in a few of our allograft recipients (4/16), $CD8^+$ T lymphocyte counts fell to normal values in 1–3 months, parallel to the disappearance of activation markers and normalization of sCD8 antigen levels. No clinical features could be correlated to the two evolutive patterns, all the more so as our definition of primary CMV infection was based on viraemia, which is usually associated with symptomatic CMV disease [20]. Antigenaemia was not available at the beginning of this study, but then confirmed CMV excretion. The two evolutive patterns were observed in kidney as well as in liver transplantation, and thus cannot be attributed to differences in organ allograft and basal immunosuppressive regimen. The two evolutive patterns were not correlated for previous treatment of rejection episodes. We also found no relation between the initial number of activated T cells and the recovery from CMV infection or the development of progressive CMV disease, as reported by Van den Berg *et al.* [21,22], but the only patient without CD8 expansion in our study developed a severe CMV disease, a further indication of the importance of activated $CD8^+$ T cells in defence against CMV [3,21].

The use of lymphocyte markers in the assessment of CMV infection has largely been centred on the well documented increase of the $CD8^+CD57^+$ T cell population, a hallmark of CMV infection [9,11–14]. We verified that the percentage of $CD57^+$ in $CD8^+$ T lymphocytes was stable at 5–15% in our seronegative transplant patients with no virological changes (Table 1). Disproportionate expansion of this subset in active CMV infection accounts for the elevation of the CD8 population, but only after a delay, so that this subset was not yet elevated at the viraemic phase of primary CMV infection, and therefore cannot account for the initial expansion of the CD8 lymphocytic population.

In contrast, our data in transplant patients with CMV reactivation or reinfection reveal the simultaneous increase of the CD8 population and of its $CD8^+CD57^+$ subset, so that half-maximum values of $CD8^+CD57^+$ cell counts are observed on average 7 days after the first viraemia (data not shown). Forman *et al.* [13] also report a peak of $CD57^+$ cells concomitant with viraemia in patients who had evidence of reactivated CMV infection after bone marrow transplantation. In addition, CMV carriers have significantly increased numbers of $CD8^+CD57^+$ compared with non-carriers [11,12]. Taken together, these observations suggest that $CD8^+CD57^+$ T lymphocytes represent 'memory' cells in CMV infection, that are rapidly mobilized in CMV reactivation but of late and progressive appearance in primary CMV infection. The function of $CD8^+CD57^+$ T cells is still poorly defined [12,23–25].

In around half of our transplant patients, CD8 expansion started with a transient increase in $CD8^+CD28^+$ T lymphocytes. The initial mobilization of the $CD8^+CD28^+$ T cell subset could represent an anti-viral cellular response, since CMV-specific cytotoxic T lymphocytes express the CD28 molecule [3]. An early but moderate peak of $CD8^+CD56^+$ and/or $CD8^+CD11b^+$ T lymphocytes was observed in a few other patients, and it could also reflect the anti-viral response [26].

Either at the second month or, in other patients, right at the onset of CMV primary infection, the expanded CD8 compartment in made up mostly of uncommon T lymphocytes which express brightly the CD8 molecule and bear T cell markers (CD3 and TCR$\alpha\beta$), but cannot be ascribed to any particular subset with the panel of antibodies tested (CD28, CD11b, CD56, CD57, CD16). Nevertheless, these cells segregate into $CD45RA^+$ or $CD45RO^+$ in the same proportion as common $CD8^+$ T cells.

Lack of detection of subset-specific markers of $CD8^+$ T lymphocytes cannot be related to their down-modulation, considering the close time interval of our follow-up analyses. The CD11b molecule is down-regulated only upon activated T lymphocytes [26], but $CD8^+CD11b^+$ T lymphocytes represent a minor CD8 subpopulation precisely when the expression of activation markers has disappeared. The acquisition of CD28 molecule by $CD8^+CD28^-$ clones is reported, but not the opposite [27,28]. We can only speculate that the $CD3^+CD8^{+\,bright}CD28^-CD11b^-CD16^-CD56^-CD57^-$ cells belong to a subpopulation rare in healthy subjects, for which no specific marker has been tested or is available, that is directly expanded as the result of a selective process related to primary CMV infection.

Our longitudinal studies indicate altogether that a persisting CD8 lymphocytosis in primary CMV infection in allograft recipients is directly related to the rate of expansion of an uncommon $CD8^+CD57^-$ subset and its progressive replacement by $CD8^+CD57^+$ T cells that are successively elicited by CMV. Two hypotheses can be made: (i) distinct CD8 subsets could follow each other during the first 6 months of primary CMV infection, with a slower expansion rate of $CD8^+CD57^+$ lymphocytes; (ii) as the CD57 antigen is a carbohydrate epitope borne by several cell adhesion molecules [29], the CD57 marker could be acquired as a result of the maturation of an (initially) $CD8^+CD57^-$ T lymphocyte subset. Whether this is the result either of a differentiation or of an activation disorder in $CD8^+$ T cell lineage is currently under investigation. Whatever the mechanism, linkage of the two phenotypes is suggested since in the few transplant patients who did not maintain a CD8 expansion after the first months, $CD8^+CD57^-$ and later on the $CD8^+CD57^+$ subsets did not expand even in percentages within $CD8^+$ cells.

CMV-specific cytotoxic precursor T cells are estimated at a frequency of 1/5000 to 1/20 000 in peripheral blood lymphocytes [2]. It thus seems unlikely that CMV-specific T cells determine the bulk of a stable CD8 expansion. Therefore, we investigated the usage of TCR Vβ genes in $CD8^+$ cells with MoAbs against specific TCR Vβ families. Although this approach could assess only a portion of the many Vβ families, we detected no bias towards the use of a particular Vβ protein by any major CD8 subset that predominates at the successive phases of CMV infection. These results are in agreement with Southern blot analysis of $CD3^+CD57^+$ lymphocytes in long-term kidney allograft recipients [30], and suggest that the selective imbalances of $CD8^+$ T lymphocyte subsets result from polyclonal recruitment.

As the disproportionate elevation of minor (in normal subjects) $CD8^+$ T cell subsets persists up to 4 years after primary CMV infection in allograft recipients, this indicates also that the combination of immunosuppressive therapy and CMV-derived factors results in the establishment of a novel homeostasis of the peripheral CD8 lymphocytic compartment during primary CMV infection.

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