Peripheral T Cell Activation in Long-Term Renal Transplant Patients: Concordant Upregulation of Adhesion Molecules and Cytokine Gene Transcription

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ABSTRACT
In renal transplant patients, the number of T cells expressing high levels of LFA-1 (LFA-1-bright) and of T cells expressing CD57 increases in response to viral infection, even if the latter is asymptomatic. Their role in long-term renal transplant patients with cytomegalovirus (CMV) antigenemia and concomitant transplant dysfunction was investigated. For this purpose, this study used triple-color flow cytometry, fluorescence-activated cell sorting of peripheral blood T cells (CD3+/LFA-1-dim or -bright and CD8+/CD57+ or CD57− subsets), and subsequent semiquantitative reverse transcription-polymerase chain reaction. Cytokine mRNA levels for interleukin (IL)-1β, IL-2, IL-4, IL-8, IL-10, tumor necrosis factor α, and interferon-gamma, as well as Granzyme A and IL-2R p55 and p75 transcripts were determined and compared in peripheral blood mononuclear cells and in separated T cell subsets. Although in patients with CMV infection and/or rejection, cytokine transcripts were readily detected and the levels in the CD3+/LFA-1-bright subsets were, by orders of magnitudes, higher than in the LFA-1-dim subset, hardly any cytokine message was found in patients without CMV infection or rejection episodes or in control subjects. The expression of Granzyme A, which is involved in cytotoxic T lymphocyte-mediated cytotoxicity, was not upregulated in LFA-1-bright T cells, which is in discordance with cytokine levels. Differences between CD57+ and CD57− T cells were limited to the IL-2R p55 mRNA, of which the former expressed significantly less than the latter. It is concluded that upon virus-induced activation of peripheral blood T cells, an effector type that is marked by high inflammatory but small cytotoxic potential is produced. The results of this study propose that these cells represent a correlate of persistent immune activation and are liable to produce graft dysfunction, although they are unable to clear the organism from virus infection because of their lack of cytotoxic potential.

Key Words: Cytomegalovirus, rejection, CD57, LFA-1, PCR

Successful renal transplantation requires effective long-term immunosuppression, which, unfortunately, compromises anti-infectious and particularly antiviral defense. Cytomegalovirus (CMV) is one agent that is typically found in renal-transplant patients. Interestingly, in these patients, CMV antigenemia is often observed in the absence of CMV disease. This does not imply, however, that CMV antigenemia is not a critical condition: clinical observations in our center have established a striking association between clinically "inapparent" CMV antigenemia and late renal graft dysfunction (1). Can this obscure association be explained? We hypothesize that non-allograft-specific activated T cells are enabled to infiltrate kidney grafts on the grounds of increased graft endothelial expression of adhesion molecules. For example, the vascular adhesion molecule 1 (VCAM-1) and the intercellular adhesion molecule 1 (ICAM-1) are upregulated in kidney grafts (2) and especially in long-term grafts (our own unpublished observation). On the other hand, the CD8+/CD57+ subset of peripheral blood T cells, which is often found to be increased in renal-transplant patients, expands in response to viral infection (3–5). These T cells, which lack CD28 expression (6), are LFA-1-bright and express high levels of CD49e (Very Late Antigen-alpha 5) (7). Although LFA-1 (CD11a/CD18) is the ligand of ICAM-1 on endothelial cells, the fibronectin receptor CD49e, which is coregulated with LFA-1 on T cells (7), has recently been implicated in T cell activation/co-stimulation by extracellular matrix proteins (ECM) and in connective-tissue transmigration along the ECM (8,9). Lack of CD28 expression, in addition, points to CTL activity (10). It seems, therefore, that despite immunosuppression, virus infection produces rather potent effector-type T cells in these patients. To establish whether CD57+ or LFA-1-bright T cells differ from CD57− or LFA-1-dim T cells in renal transplant patients with virus antigenemia and/or graft rejection in such a way that their potential to cause tissue damage is greatly increased, we decided to study in vivo cytokine mRNA expression in freshly isolated peripheral blood mononuclear cells (PBMC) and in T cells from...
renal transplant patients with CMV infection or graft rejection and in patients with none of these complications. Fluorescence-activated cell sorting was used to separate T cell subpopulations (CD8+/CD57+ or CD57− and CD3+/LFA-1-bright or -dim) and a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique was performed to compare the amounts of mRNA for the following cytokines and other products in each subpopulation (interferon-gamma [IFN-γ], tumor necrosis factor α [TNF-α], interleukin [IL]-1β, IL-4, IL-8, IL-10, IL-2R p55 and p75, and Granzyme A). Our results show that in renal transplant patients with CMV infection or rejection, PBMC express readily detectable amounts of cytokine mRNA, whereas a cytokine message was not detected or was only just above the detection threshold in the absence of such complications. The sorting experiments show that cytokine mRNA is almost exclusively found in LFA-1-bright T cells, whereas CD57+ expression on CD8+ T cells does not seem to identify an individual subset with increased cytokine gene expression.

METHODS

Subjects

Three groups of patients (A, B and C) and one control group were examined. The groups were compiled and examined at different times.

Group A (unsorted PBMC were examined with respect to mRNA contents). Group A comprised 12 renal-transplant patients without CMV antigenemia or positive serum IgM and with no current episode of graft rejection. Immunosuppression included triple-drug therapy in eight of the 12 patients, i.e., cyclosporin A (CsA), prednisolone or methylprednisolone (Pred), and azathioprine (Aza). Pred/Aza and CsA/Aza were both used in two of the 12 patients. Graft survival time was between 5 and 22 yr, age ranged from 22 to 51 yr (mean ± SE, 36 ± 3.33). Four patients were female and eight were male.

Group B (unsorted PBMC and sorted CD57+ or CD57− and CD8+ T cells were examined with respect to mRNA contents). Group B included eight kidney transplant patients, all with clinically asymptomatic CMV infection (virus antigenemia and/or positive serum IgM) and histology-proven late acute rejection (according to the Banff Classification). Immunosuppression included triple-drug therapy in two of the eight patients, CsA/Aza in three of the eight, Pred/Aza in one of the eight, and CsA/Pred in two of the eight. Graft survival was between 6 months and 10 yr and age ranged from 33 to 65 yr (mean ± SE, 48.07 ± 4.39). Three patients were female and five were male.

Group C (sorted LFA-1-bright or LFA-1-dim CD8+ T cells were examined with respect to mRNA contents). Group C was composed of ten long-term renal-allograft recipients with clinically asymptomatic CMV infection (virus antigenemia and/or positive serum IgM). Histologic studies identified two of the ten as late acute rejection (according to the Banff Classification), and eight of ten had signs of graft infiltration only. Immunosuppression included triple-drug therapy in eight of the ten patients; one of the ten received CsA and Pred, and one of the ten received Pred only. Graft survival was >2 yr in all patients. Age ranged from 20 to 56 yr (mean ± SE, 40.9 ± 4.28 yr). Five patients were female and five were male.

Control Subjects

Age ranged from 20 to 54 yr (mean ± SE, 32.5 ± 4.16 yr). All control subjects were healthy volunteers. One subject was female and six were male.

Sample Preparation

Mononuclear cells were prepared by standard FICOLL-HYPAQUE (Pharmacia, Uppsala, Sweden) density gradient centrifugation from 20 mL citrated peripheral blood. Cells were washed twice in phosphate-buffered saline and then resuspended in phosphate-buffered saline containing 2% fetal calf serum (vol/vol) and 0.1% sodium-azide (wt/vol).

Flow Cytometry and Fluorescence-Activated Cell Sorting

For analysis regarding expression of CD28 and CD57 on CD8+ T cells, cells were stained with FITC-labeled anti-CD28 (Dianova, Hamburg, Germany), phycoerythrin (PE)-labeled anti-CD28 (Becton Dickinson, Heidelberg, Germany), and PerCP-labeled anti-CD8 according to the manufacturers' instructions.

For separating LFA-1-bright and -dim subsets, the cell suspension was incubated with FITC-labeled murine anti-human CD11a (LFA-1-alpha chain, Dianova) and PerCP-labeled murine anti-human CD3 (Becton Dickinson) monoclonal antibodies for 30 min at 4°C. For separating CD57+ and CD57− subsets, cells were stained with FITC-labeled anti-CD57 antibody and PE-labeled anti-CD8 antibody (both from Dianova). First, lymphocytes were gated in scatterlight, then T cells were divided into the subsets of interest according to their degree of marker expression. Cell sorting was carried out with an EPICS-C cell sorter (Coulter Electronics, Krefeld, Germany). After cell sorting, 50 μL of the original suspension and the two fractions obtained were counterstained with either PE-labeled anti-human CD14 (Dianova) or TRI-COLOR-labeled murine anti-human CD14 (MEDAC, Hamburg, Germany) to estimate the number of monocytes. Reanalysis was carried out with a FACscan (Becton Dickinson) using triple-color fluorescence. The contamination of LFA-1-dim T cells with LFA-1-bright T cells and vice versa was lower than 1%. The number of monocytes in all samples was less than 3% and the degree of contamination with monocytes was the same in LFA-1-bright cells as in LFA-1-dim cells.

Semiquantitative RT-PCR

Unseparated PBMC or T cell subsets separated according to CD57 expression (±) or LFA-1 expression (bright/dim) were centrifuged and resuspended in lysis buffer. Total RNA was extracted and reverse-transcribed as explained elsewhere (11). A cDNA equivalent of approximately 5 ng cellular RNA was taken for each PCR analysis. Identity of PCR products from cDNA was verified by restriction analysis with indicative of the expected amplified sequence. Two synthetic multispecific competitor gene fragments—the control fragments (CF)—containing the 5′ and 3′ primers were constructed and relative quantitation of specific cDNA was carried out by competitive RT-PCR. This method was described in detail elsewhere (11, 12). In brief, varying known amounts of the above mentioned CF were added to unknown but constant amounts of sample cDNA to compete for amplification with specific primers. The proportions of PCR prod-
ucts amplified from CF and target cDNA were estimated after separation on 1.5% agarose gel by measuring the intensity of ethidium bromide luminescence with a CCD image sensor and analyzing the data with the EASY program (Herolab, Wiesloch, Germany). The relative concentration of the pertinent cytokine cDNA in each sample cDNA was estimated from the concentration of CF DNA that achieved equilibrium between its own amplification and the amplification of the target cDNA. It was expressed in arbitrary units. One AU was defined as the lowest concentration of CF that yields a detectable amplification product given one particular primer pair and the PCR conditions used.

To correct for variations across different preparations, all samples to be compared were adjusted to contain equal input cDNA concentrations. This was based on their GAPDH "housekeeping gene" cDNA content, which was determined by competitive PCR. We then estimated the cDNA content for the cytokines (IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-8, IL-10), Granzyme A, IL-2R p55 (IL-2Ra), and p75 (IL-2Rβ) in these adjusted samples and compared the results obtained for the two subpopulations.

To simplify the PCR data, differences between LFA-1-bright and LFA-1-dim subsets were expressed as the ratio of mRNA expression in the LFA-1-bright/mRNA expression in the LFA-1-dim subset. Because of the use of dilutional steps, the given maximum differences between the two subsets regarding a particular product represent a "minimum" of the maximal difference if the product was not detected in LFA-1-dim T cells.

RESULTS

Cytofluorometric Characterization of CD8+ T Cells in Patients

As described in the Methods section, we selected patients with clinically asymptomatic CMV infection and/or rejection. These patients are characterized by an expansion of CD8+ T cells expressing high levels of LFA-1. The relationship between LFA-1-expression, CD57 expression, and CD28 expression on peripheral blood CD8+ T cells in one patient (Group C) is shown in Figure 1. CD28− CD8+ T cells are LFA-1-bright. Almost all CD57+ T cells are CD28− (and LFA-1-bright). Essentially the same picture was found in the other patients.

To establish whether cytokine gene expression in renal-transplant patients suffering from graft rejection and CMV infection (Group B) was different from cytokine expression in renal-transplant patients with neither rejection nor CMV infection (Group A), unsorted PBMC were studied before we separated T cell subsets. We found that cytokine mRNA was readily detectable in Group B, whereas in Group A and in healthy control subjects, no cytokine mRNA was detected or the amounts were, by orders of magnitude, less (levels close to detection threshold) (Table 1).

As a consequence, looking for the source of increased cytokine expression was promising only in those patients with CMV infection or late acute rejection (readily detectable cytokine message, Groups B and C), the other group of patients (no rejection episode, no CMV infection) did not seem to warrant separation of subsets (cytokine message barely detectable, Group A). Group B was studied with respect to differences in cytokine gene expression between CD57+ and CD57− CD8+ T cells (only CD8+ T cells were considered because CD57 expression on CD4+ T cells was negligible) and Group C was studied with regard to such differences between LFA-1-bright and -dim CD3+ T cells. The latter provided the most striking results.

Figure 1. The typical distribution of CD11a, CD28, and CD57 expression on CD8+ (high) T cells in a renal transplant patient is shown by triple-color flow cytometry. CD8+/CD28− T cells are CD11a-bright (A) and CD57 expression is essentially limited to the CD28− subset (B). For both panels, CD8-high T cells were electronically gated in a sideward-scatter/FL-3 dot plot. Diagrams show log fluorescence intensity.
TABLE 1. Cytokine gene expression in peripheral blood mononuclear cells in renal transplant patients with (B) and without (A) cytomegalovirus (CMV) infection/rejection (rej.) and healthy control subjects a, b

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Renal Transplant Patients</th>
<th>Healthy Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with CMV/Rej. (B) (N = 8)</td>
<td>w/o CMV/Rej. (A) (N = 12)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>95 (8 to 310) 0 (0 to 2) c</td>
<td>0 (0 to 1) 0 (0 to 1)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>57 (0 to 315) 1 (0 to 15) d</td>
<td>0 (0 to 1) 0 (0 to 1)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>53 (5 to 110) 5.5 (1 to 55) d</td>
<td>0.5 (0 to 5) 0.5 (0 to 1)</td>
</tr>
<tr>
<td>IL-4</td>
<td>70 (1 to 105) 1 (0 to 6) d</td>
<td>0.5 (0 to 1) 0.5 (0 to 1)</td>
</tr>
<tr>
<td>IL-8</td>
<td>29 (1 to 110) 25 (0 to 55)</td>
<td>1 (0 to 5) 1 (0 to 5)</td>
</tr>
<tr>
<td>IL-10</td>
<td>55 (0 to 110) 0 (0 to 5) d</td>
<td>0 (0) 0 (0)</td>
</tr>
</tbody>
</table>

a Values given are median and range (arbitrary units). Differences between Groups A and B were tested for statistical significance using the Mann-Whitney test for unpaired samples.

b IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor α; IL, interleukin.

c P < 0.01.
d P < 0.05.

Cytokine Gene Expression Is Almost Exclusively Found in the LFA-1-Bright T Cell Subset

Differences between LFA-1-dim and LFA-1-bright subsets were clearly noticeable on the gels before quantification (amplification in the absence of control fragment) (Figure 2). Cytokine mRNA levels for all detected cytokines (TNF-α, IFN-γ, IL-10, IL-8, IL-4, IL-2 and IL-1β) were clearly higher in LFA-1-bright T cells than in LFA-1-dim T cells in all but one instance (IL-1β in one patient). Semiquantification using competitive RT-PCR confirmed that these differences were mostly of one or several orders of magnitude (between 100- and 2000-fold) (see Table 2 for details). IL-4 and IL-10 mRNA expression was only detected in the transplant recipients suffering from histologically proven late acute rejection in addition to CMV infection (Figure 2). As with the other cytokines, gene expression was restricted to the LFA-1-bright T cell subset (factor, >1000). Moreover, IL-2 gene expression was only found in the patient with late acute rejection who was not on CsA. It was also limited to the LFA-1-bright T cell subset (Table 2).

The expression levels of both IL-2R chains (p55 and p75) and of Granzyme A were not essentially different.

Figure 2. Cytokine gene expression in peripheral blood mononuclear cells (PBMC) from two renal-transplant patients with cytomegalovirus infection and late acute graft rejection proven by histology (Group C). Reverse transcription-polymerase chain reaction (RT-PCR) shows much higher amounts of cDNA product in the LFA-1-bright subset (differences not quantified). Amounts of input cDNA were adjusted by semiquantitative RT-PCR (using GAPDH) and the number of amplification cycles for each subset were identical. A and B, patients; I, LFA-1-bright; II, LFA-1-dim. IL2β is the p75 β-chain of the IL-2-receptor.
TABLE 2. Cytokine mRNA content: comparison between LFA-1-bright and LFA-1-dim T cell subsets in renal transplant patients with cytomegalovirus (CMV) infection only or CMV infection and rejection (Group C)a

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Max Ratiob</th>
<th>N</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>&gt;500c</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>IL-1β</td>
<td>&gt;2000d</td>
<td>10</td>
<td>150</td>
</tr>
<tr>
<td>TNF-α</td>
<td>&gt;1000d</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>IL-2</td>
<td>&gt;100</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>IL-4</td>
<td>&gt;2000</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>IL-8</td>
<td>&gt;500d</td>
<td>10</td>
<td>500</td>
</tr>
<tr>
<td>IL-10</td>
<td>&gt;2000</td>
<td>2</td>
<td>ND</td>
</tr>
</tbody>
</table>

a All patients had signs of CMV infection, in addition to which two patients were suffering from histology-proven rejection. There is more cytokine mRNA in LFA-1-bright than in LFA-1-dim T cells. P was calculated using the Wilcoxon test for paired samples.
b The difference between the subsets is expressed as ratio (mRNA in LFA-1-bright T cells/mRNA in LFA-1-dim T cells).
c N denotes the number of patients in whom the pertinent cytokine mRNA was detected.
d P < 0.01.
e P < 0.05.
f This patient was not on cyclosporin A.
g These two patients were suffering from histology-proven graft rejection in addition to CMV infection.

TABLE 3. Difference in IL-2R p55 and Granzyme A mRNA content between LFA-1-bright and LFA-1-dim CD3+ T cells in renal-transplant patients and control subjectsa

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patientsa (N = 10)</th>
<th>Control Subjects (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratiob (Range)</td>
<td>Ratio (Range)</td>
</tr>
<tr>
<td>IL-2R p55</td>
<td>1 (1 to 100)</td>
<td>1 (0.5 to 2)</td>
</tr>
<tr>
<td>IL-2R p55</td>
<td>1.5 (0.5 to 100)</td>
<td>1 (1 to 5)</td>
</tr>
<tr>
<td>Granzyme A</td>
<td>2 (0.5 to 100)</td>
<td>5 (1 to 50)</td>
</tr>
</tbody>
</table>

a Differences between patients and control subjects were statistically not significant (Mann-Whitney test for unpaired samples).
b Patients were suffering from CMV infection alone or CMV infection and rejection (Group C).

differences between the subsets is expressed as ratio (mRNA in LFA-1-bright T cells/mRNA in LFA-1-dim T cells). The median is given.

in the two subsets (Group C) (Table 3). Nevertheless, Granzyme A expression seemed higher in the LFA-1-bright cells obtained from patients than in the LFA-1-bright cells from control subjects (Table 4).

CD57+/CD8+ T Cells Have the Phenotype of Nonproliferating Effector T Cells

The CD8+/CD57+ T cell subset is the most prominent example of a T cell subpopulation that expands in response to viral infection. Analysis of separated CD57+ and CD57− CD8+ T cells (Group B) revealed that both subsets expressed similar levels of mRNA for the previously mentioned cytokines (not shown). However, with regard to cytokine receptor expression, significantly less mRNA for the p55 IL-2R transcript was found in CD8+/CD57+ T cells (Figure 3).

**DISCUSSION**

Two principal conclusions can be drawn from our data: (1) T-cell cytokines are almost exclusively produced in LFA-1-bright T-cells, and (2) that this only occurs after immune activation (comparatively negligible amounts or absence of cytokine mRNA in patients without CMV infection or signs of rejection, and in control subjects).
Figure 3. Quantification of the IL-2R p55 gene expression in CD57+/CD57- T cell subsets from two renal transplant patients with cytomegalovirus infection and late acute graft rejection (Group B). Quantification was done with semiquantitative RT-PCR using a control fragment (CF) competing with the sample cDNA for the IL-2R p55 primer. The left lane shows PBMC, the middle lane CD8+/CD57+ T cells, and the right lane CD8+/CD57- T cells. With increasing dilutions of the CF (indicated by I, II, and III at the bottom of the figure), there is increasing cDNA amplification.

It is suggested by two patients in Group A who suffered from histology-proven late acute rejection (in addition to virus infection) that differential cytokine expression occurs depending on the activating event; however, it was limited to LFA-1-bright T cells. Type 2 cytokine expression (IL-4 and IL-10) was found in addition to the otherwise prevalent Type 1 cytokine expression. In addition, IL-2 mRNA was detected in one of these two patients, who was not being treated with CsA.

Our results clearly show that LFA-1-bright peripheral blood T cells, which seem well equipped to leave the blood circulation and penetrate into sites of inflammation (increased expression of the adhesion molecules LFA-1, CD49e, and others), are also endowed with the properties to “take action” where needed (upregulated cytokine production). Although in some transplant patients with clinically asymptomatic CMV infection up to 80% of CD8+ T cells belong to the LFA-1-‘bright’ population, it has not been determined yet whether these are recirculating virus-specific T cells or allospecific. Irrespective of what their target is, it may be concluded that CMV infection in transplant patients induces T effector cells liable to cause tissue damage because of their heightened capacity for tissue infiltration and their inflammatory potential.

Although there was a marked difference between LFA-1-bright and -dim T cells regarding cytokine gene expression, no such difference was found with respect to Granzyme A, a serine protease involved in CTL-mediated cytotoxicity. It seems, therefore, that in long-term transplant patients, LFA-1-bright T cells have high inflammatory potential, although their cytotoxic potential is not or is only marginally increased. By contrast, in vitro-generated virus- or allospecific cytotoxic T cells, as well as graft-infiltrating T cells during early acute cellular rejection, show markedly
increased gene expression of perforins and granzymes (13,14). The lack of upregulation of this "secretory" pathway of CTL-mediated cytotoxicity in our patients is probably a consequence of long-term immunosuppression. This is supported by the recent recording of intragraft upregulation of inflammatory cytokines, but not granzymes, during "late" acute rejection (15), which may also explain why the latter is generally more protracted than "early" acute rejection.

The role of activated effector-type T cells in long-term allograft recipients with clinically asymptomatic CMV infection is still not fully understood. We speculate that the expansion of Type 1 cytokine-expressing effector T cells prevents the virus from spreading and, thus, the development of CMV disease (which is rare in these patients). Because of the lack of cytotoxic potential, however, the organism is not cleared from reactivated virus. The result of this may be characterized as a "stalemate" in that virus infection and immune activation persist. In the long run, persistent activation of cytokine-producing T effector cells may cause adverse effects such as fatigue or graft injury (1).

Notably, the levels of IL-2R p55 expression in LFA-1-bright and in LFA-1-dim T cell subsets were not significantly different. The expression of IL-2R p55 in LFA-1-dim T cells indicates that this subset contains a proportion of freshly activated but "not-yet-effector" cells. Once effector function is acquired (LFA-1-bright T cells), terminal differentiation with loss of proliferative capacity may occur (lack of IL-2R p55 mRNA associated with CD57 expression and loss of CD28 expression on LFA-1-bright T cells). Hence, CD57 (or lack of CD28) may be a marker of terminal differentiation yet makes no additional statement about the cytokine profile of the concerned population.

Unlike other researchers, who derive their data regarding resting and activated human T cells from in vitro models, we consider the molecular "blueprint" (i.e., cytokine gene transcription) of cytokine production in freshly isolated human T cells. We believe that this provides a better reflection of reality, nevertheless, we understand that post-transcriptional regulation of cytokine levels may also take place. In addition to looking at peripheral blood T cell subsets, we will look more closely at intragraft events in the future: preliminary data suggest that in patients with virus infection-associated graft injury, LFA-1-bright T effector cells accumulate in the graft.

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