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Analysis of immune cells draining from the abdominal cavity as a novel tool to study intestinal transplant immunobiology

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Summary

During intestinal transplant (ITx) operation, intestinal lymphatics are not reconstituted. Consequently, trafficking immune cells drain freely into the abdominal cavity. Our aim was to evaluate whether leucocytes migrating from a transplanted intestine could be recovered from the abdominal draining fluid collected by a peritoneal drainage system in the early post-ITx period, and to determine potential applications of the assessment of draining cellular populations. The cell composition of the abdominal draining fluid was analysed during the first 11 post-ITx days. Using flow cytometry, immune cells from blood and draining fluid samples obtained the same day showed an almost complete lymphopenia in peripheral blood, whereas $CD3^+CD4^+CD8^-$, $CD3^+CD4^-CD8^+$ and human leucocyte antigen D-related $(HLA\text{-}DR)^+CD19^+$ lymphocytes were the main populations in the draining fluid. Non-complicated recipients evolved from a mixed leucocyte pattern including granulocytes, monocytes and lymphocytes to an exclusively lymphocytic pattern along the first post-ITx week. At days 1–2 post-Itx, analysis by short tandem repeats fingerprinting of $CD3^+CD8^+$ sorted T cells from draining fluid indicated that 50% of cells were from graft origin, whereas by day 11 post-ITx this proportion decreased to fewer than 1%. Our results show for the first time that the abdominal drainage fluid contains mainly immune cells trafficking from the implanted intestine, providing the opportunity to sample lymphocytes draining from the grafted organ along the post-ITx period. Therefore, this analysis may provide information useful for understanding ITx immunobiology and eventually could also be of interest for clinical management.

Keywords: abdominal cavity drainage, immune cells, intestinal transplant, lymphocytes

Introduction

Small bowel transplantation (Tx) has become an accepted clinical option to treat patients with intestinal failure and total parenteral nutrition (TPN) life-threatening complications [1,2]. Availability of new immunosuppressant protocols and improvement in post-operative patient management have decreased mortality and graft loss, improving survival. In spite of this progress, acute cellular rejection remains as one of the major threats for intestinal transplant patients [3,4], especially in the early post-Tx period when reduction of immunosupression may be needed due to infectious or surgical complications. Post-Tx immunological events are common after intestinal transplant (ITx), as the small bowel harbours a high number of immune cell populations [5]. This increases the possibilities of undergoing allogenic or non-specific immune activation in the context of ITx leading to acute cellular rejection episodes, which have been described as occurring with high frequency during the follow-up of ITx patients [6,7].

At the end of the engraftment, ITx provides high accessibility to biological material derived from the transplanted organ, giving a unique opportunity to monitor biological processes ongoing on the grafted organ directly or indirectly. The ileostomy provides direct access to the graft, being used routinely for histological follow-up for graft rejection detection, allowing sampling of intestinal content for clinical or research purposes. Furthermore, in the early post-transplant

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period an abdominal drain is used for monitoring post-operative abdominal bleeding or the presence of bile or intestinal content in the peritoneal cavity.

During the surgical procedure for isolated ITx, blood circulation can be restored using a vascular anastomosis to the mesenteric circulation or to the aorta and cava vein (systemic circulation). However, the mesenteric lymphatic vessels are not reconstructed in any case. Under normal physiological conditions, mesenteric vessels collect the leucocytes migrating out of the intestinal mucosa [5]. We hypothesized that cell analysis of the peritoneal cavity drainage allows the assessment of immune cells migrating directly out of the graft through the intestinal lymphatic vessels.

In the present report, we analysed the leucocyte populations present in the fluid collected by the abdominal drain left in the early post-operative period in a series of isolated ITx recipients. To our knowledge, there has been no previous report showing the feasibility, value and potential clinical benefits of performing this type of analysis in post-Tx clinical practice. The data presented here indicate that this approach may provide a deeper insight into the biology of immune cells migrating out of the graft, improving our understanding of immune processes that take place in the early post-transplant period.

Materials and methods

Patients and case reports

One adult and five paediatric patients presenting short bowel syndrome (SBS) and lack of central venous accesses as indication underwent isolated ITx between December 2007 and November 2008 and were enrolled in this study.

Patient 1 was a 53-year-old male, with SBS secondary to intestinal ischaemia. Patient 2 (a 9-year-old boy), patient 3 (a 4-year-old boy), patient 5 (a 9-year-old boy) and patient 6 (a 6-year-old girl) presented SBS secondary to volvulae. Patient 4 was a 3-year-old girl with SBS secondary to gastroschisis. Patients 2 and 4 were engrafted with portal drainage. No severe intra-operatory complications were observed. All patients received ABO-compatible grafts (1 mismatch O-A); all had cross-matches performed prospectively; only patient 6 was transplanted with a positive B cell cross-match. The major clinical details, immunosuppressive treatment and major clinical events in the first 20 days post-ITx are summarized in Table 1. Only patient 1 required an exploratory laparotomy due to post-endoscopic bleeding and questionable perforation. The findings were negative for perforation and positive for peritonitis, but with negative cultures from the material obtained.

The study was approved by the Institutional Review Board of Favaloro Foundation. All six patients were informed and signed an agreement to participate in the study.

Collection of draining fluid

Isolated ITx can be performed using systemic or portal drainage; in both cases, the mesenteric lymphatic vessels are not reconstructed (Fig. 1a,i), leaving them draining freely into the peritoneal cavity after blood circulation is restored (Fig. 1a,ii). Before closing the abdominal wall, a silicon drainage that recovers fluid from the peritoneal cavity is placed close to the mesenteric border (Fig. 1a,iii). The sampling of draining fluid started from post-Tx day 0 and continued for 2–3 weeks, depending on the clinical condition. Draining fluid was aspirated into a closed receptacle, emptied when full, and the volume and quality of the liquid were recorded systematically. Samples for cell analysis were collected overnight (from midnight to 8 a.m.).

Cell count in different fluids

Whole blood and draining fluid cell counts were assessed with an automated cell counter (Cell-dyn 3200; Abbott Diagnostics, Abbott Park, IL, USA) every second day as part of the clinical follow-up.

Cell purification

Draining fluid samples were filtered through an 80-μm filter mesh (BD Biosciences, San Jose, CA, USA) in 50 ml Falcon

Table 1. Detailed immunosupressive regimen and clinical complications in the early post-TX period.

			Immunosupression			
Patient	Age	Diagnosis before ITx	Donor	Induction	Maintenance	Complications after ITx
1	53	SBS secondary to intestinal ischemia	Thymoglobulin	Anti-CD25	Tacrolimus, MMF, steroids	Peritonitis, intestinal bleeding
2	9	SBS secondary to volvulae		Thymoglobulin	Tacrolimus, MMF, steroids	
3	4	SBS secondary to volvulae	Thymoglobulin	Anti-CD25	Tacrolimus, MMF, steroids	
4	3	SBS secondary to gastroschisis	Thymoglobulin	Anti-CD25	Tacrolimus, MMF, steroids	Peroral herpes lesions
5	9	SBS secondary to volvulae	Thymoglobulin	Anti-CD25	Tacrolimus, MMF, steroids	
6	6	SBS secondary to volvulae	Thymoglobulin	Thymoglobulin	Tacrolimus, MMF, steroids	Pancreatitis, abdominal collection

Itx: intestinal transplant; MMF: mycophenolate mofetil; SBS: short bowel syndrome.

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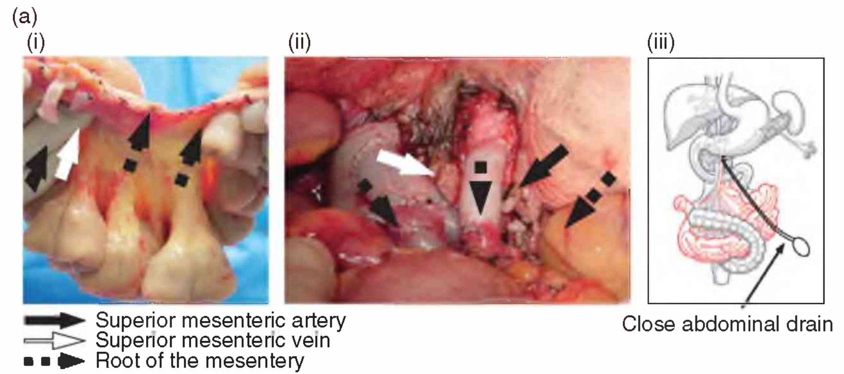

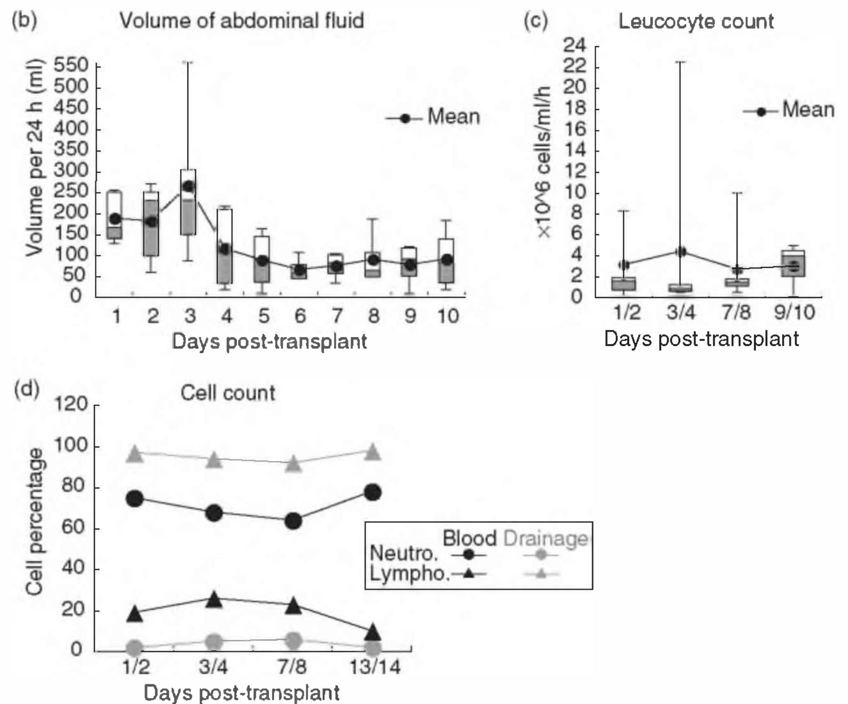

Fig. 1. Analysis of draining fluid after isolated intestinal transplant. (a) Graft after backtable surgery of isolated intestinal transplant (Itx). (i) The root of mesentery is dissected, isolating mesenteric artery and vein. The disruption of lymphatics in the root of mesentery allows cells flowing through the lymph to leak into the peritoneal cavity. (ii) Reconstitution of blood flow during implantation is shown. This allows normal blood flow to the graft and minimizes blood release into the peritoneal cavity. Lymphatic vessels dissected in the root of mesentery are not ligated. (iii) A schematic draw of the collection of draining fluid with a Jackson–Pratt drainage system. (b) Volume of draining fluid collected daily shown as a box-plot indicating 25th and 75th percentiles, the mean and the standard deviation (s.d.) of values recorded for the six patients analysed. (c) Total number of leucocytes measured by automated cell counter in the draining fluid, represented in box-plot, as indicated in (b). (d) Comparative cell count measured by automated cell counter of neutrophils and lymphocytes between peripheral blood and draining fluid of a single patient (patient 3) at different days post-ITx.

tubes and centrifuged at 400 ***g*** for 10 min at 4°C. To lyse erythrocytes, the cell pellet was resuspended in an adequate volume of approximately 10 ml of ammonium chloride lysing solution (ACK) and incubated for 5 min at room temperature. Fifteen ml of phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+} containing 0·1% sodium azide and 2% fetal bovine serum (FBS) (PAA, Pasching, Austria) [fluorescence activated cell sorter (FACS) buffer] were added and mixed gently. The cell suspension was centrifuged at 300 ***g*** for 5 min. Supernatant was discarded and the cell pellet was washed with 5 ml of FACS buffer, centrifuged and resuspended in 1 ml of FACS buffer. Cells were counted using a counting chamber.

Flow cytometry

First, $0·5 \times 10^6$ cells/tube were incubated with inactivated human serum to block Fc receptors. Cells were then incubated with conjugated specific antibodies: anti-CD3 (UCHT1), anti-CD4 (RPA-T4), anti-CD8 (RPA-T8; eBioscience), anti-CD19 (HIB19) anti-CD56 (MEM-188), anti-CD11b (CBRM1/5), anti-CD11c, anti-CD14 (M5E2), anti-CD86 (FUN-1), anti-human leucocyte antigen D-related (HLA-DR) (G46-6) purchased from BD Biosciences for 20 min at 4°C in the dark. Cells were washed and analysed with a BD FACSCalibur™ flow cytometer (BD Biosciences). Analysis was performed using CELLQuest™ (BD Biosciences) and FlowJo (Tree Star Inc., Ashland, OR, USA) software.

Cell sorting

A total of 1×10^7 cells were stained with anti-CD3 and anti-CD8 antibodies for 30 min at 4°C in the dark. Cells were washed and the pellet was resuspended in 600 µl FACS buffer. The sorting advice for the BD FACSCalibur™ flow

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cytometer (BD Biosciences) was used. Exclusion mode was selected, $CD3^+CD8^+$ cells gated and 100 000 cells were sorted for genomic DNA purification.

Genomic DNA purification for analysis of percentage of donor *versus* recipient cells

Genomic DNA was purified from sorted cells by a DNA purification kit (Machery-Nagel, Duren, Germany). Short tandem repeat (STR) markers were amplified and analysed using PowerPlex® 16 System (Promega, Madison, WI, USA), using standard conditions following the manufacturer's protocol. PCR amplification was performed using a GeneAmp® PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA). PCR products were analysed using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). Allele discrimination was performed using GeneMapper® ID version 3·2 software. The percentage of cell population coming from donor or recipient was calculated as average number of the percentage of each single peak compared to individual receptor and donor samples.

Correlation with clinical events

Patient records were analysed retrospectively, aiming to correlate cell population analysis with the most relevant clinical events observed in each patient.

Results

Collection of abdominal draining fluid

The volume of abdominal fluid collected in each patient varied daily and we observed interpatient variability (Fig. 1b). However, the general observed tendency is that fluid volume decreased during the post-operative period until the drain was removed. On average, during the first 3 days post-ITx, 210 ± 43 ml of abdominal fluid were collected, while the average of collected fluid from days 6–10 was 78 ± 8 ml (Fig. 1b). A sample of draining fluid was analysed daily to obtain differential counts of white blood cells (WBC). The number of red blood cells in the fluid allowed us to estimate blood contamination. Samples with more than 3×10^4 red cells/μl were discarded. Despite variations observed in each patient and on each post-operative day (Fig. 1c and d), a significant number of leucocytes ($2{\cdot}7 \pm 0{\cdot}9 \times 10^6$ cells/ml) were recovered. When peripheral blood cellularity was compared to the cell count in the abdominal fluid, a different cellular composition was observed within the same patient/day (Fig. 1d). Although lymphopenia (lymphocyte percentage 14 ± 9%, in all blood samples analysed) was observed in peripheral blood, lymphocytes constituted the most prevalent cells in draining fluids (66 ± 37%, in all peritoneal fluid samples analysed). This finding was seen repeatedly along the follow-up.

Analysis of cell populations in abdominal draining fluid

To address the question of which cell populations are present in the draining fluid, flow cytometry analysis was performed using a panel of specific antibodies against surface markers. Although the number of cells in the different gates and the FSC/SSC plot can vary during the post-operative sampling period, two main patterns during the follow-up of patients without a clinical event could be defined (Fig. 2b). The pattern that includes cells present in R1, R2 and R3 was observed commonly during the first 3 days post-transplant (pattern 1, Fig. 2a). The pattern constituted by lymphocytes (R1) was detected mainly from post-Tx days 3–5 (pattern 2, Fig. 2b) until removal of the drainage. The main lymphocyte subsets detected in R1 for either patterns 1 or 2 were $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells and $CD19^+HLA\text{-}DR^+$ B cells (Fig. 2c and d). Furthermore, R1 contained, at a very low percentage (usually less than 2%), $CD3^-CD56^+$ natural killer (NK) cells and $CD3^+CD56^+$ T cells (data not shown). When pattern 1 was observed, the main population (>80%) detected in R2 was positive for the specific monocyte/macrophage marker CD14 and for CD11c (Fig. 2e). This subset was characterized further as $HLA\text{-}DR^{high}$ (Fig. 2f), $CD11b^{low}$ (Fig. 2g) and CD86-positive (Fig. 2h), which might correspond to activated macrophages. The granulocytes (R3) were not characterized further. It should be emphasized that different clinical events, such as infections or intestinal obstructions, could be accompanied by a modification in the pattern of draining cell populations detected by flow cytometry. We observed a reappearance of gates R2 and R3 in all patients (three cases) with clinical events (data not shown). However, more cases should be analysed to conclude definitively that a correlation exists between clinical findings and changes in the abdominal fluid leucocyte count.

Progress of lymphocyte population over time

Analysis of the main lymphocyte populations along the early follow-up of each of the six patients reflects some general trends in the ongoing of the transplanted small bowel. As can be seen in Fig. 2i, despite some variability in the percentage of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells detected, both subsets remained as the major populations during the first 10 days post-ITx. B cells, defined as $CD3^-CD19^+HLA\text{-}DR^+$, tended to decrease in the first 2 days post-ITx from 30–50% to fewer than 10% of R1. Individual variation in the percentage of the B cell population observed may reflect particular clinical situations.

Donor *versus* recipient contribution to the lymphocyte populations from collected fluid

As a proof-of-principle, we wanted to address the following questions: how much of the drained $CD8^+$ T cells were still

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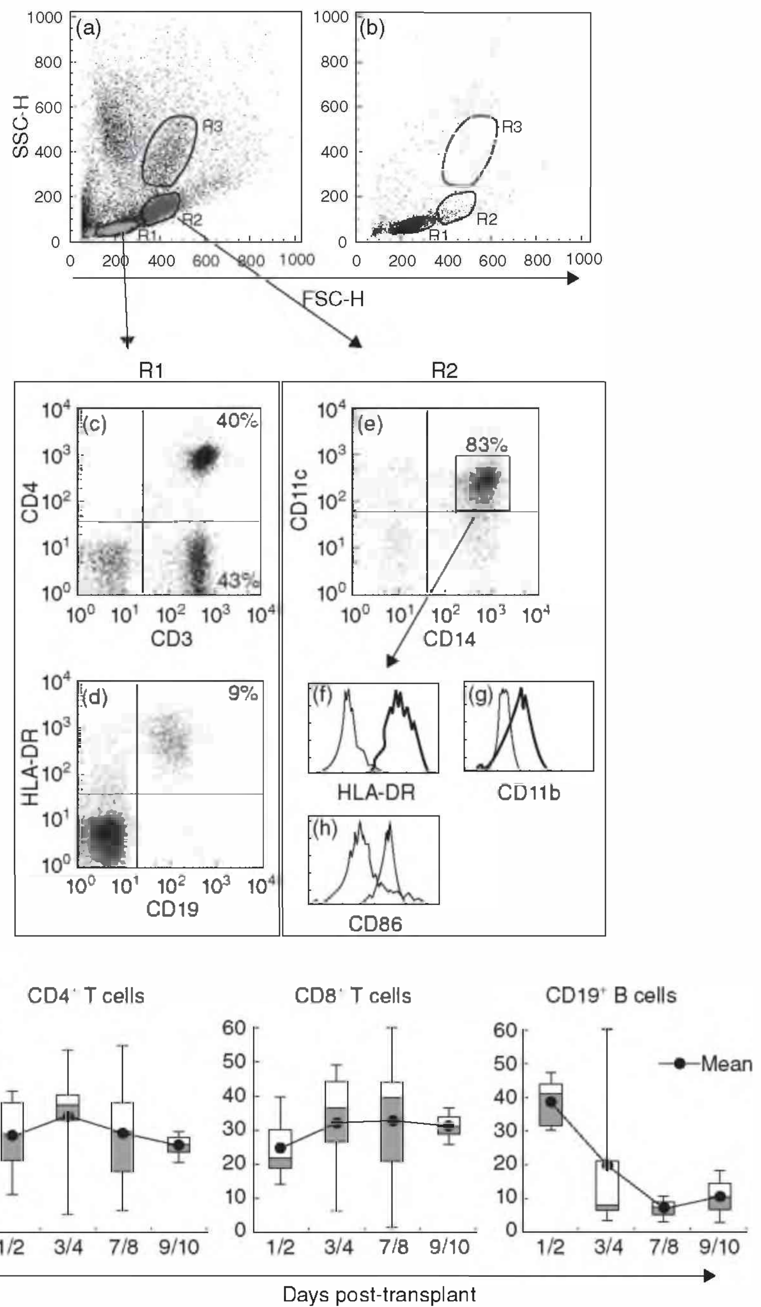

Fig. 2. Flow cytometry analysis of peritoneal draining fluid. (a) Typical forward-scatter (FSC) side-scatter (SSC) dot-plot pattern observed in the first 1–3 post-operative days. Lymphocyte gate (R1, 60%), monocyte gate (R2, 6%) and granulocyte gate (R3, 2·3%) are indicated. (b) Dot-plot representation observed after days 3–5 post-intestinal transplant (Itx), containing mainly cells in the lymphocyte gate (R1, 80%), whereas no clear populations are detected in R2 (1%) and R3 (0·2%). In both plots, 50 000 cells were acquired. Analysis of the lymphocyte gate showing mainly $CD3^+CD4^+$ or $CD3^+CD4^-$ T cell populations (c). This latter population was mainly $CD3^+CD8^+$, as detected by independent staining (not shown). $CD19^+$ human leucocyte antigen D-related $(HLA-DR)^+$ B cell population (d). Cells in the monocyte gate are positive for CD11c and CD14 (e). Histograms for the analysis of HLA-DR (f), CD11b (g) and CD86 (h) on the $CD14^+$ $CD11c^+$ gate are depicted. Isotype controls are included in each histogram. Data analysis shown corresponds to two draining samples of the same patient. Flow cytometric analyses were conducted on samples from all patients. (i) Analysis of lymphocyte subsets over time by flow cytometry. Relative percentages of $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ B cells are shown as a box-plot and the mean as symbol. Time of follow-up ranges from days 1 to 10 post-ITx.

from the donor; at which day did the recipients' lymphocytes appear in the fluid; and how their relative proportion varied along the post-operative period analysed.

To answer these questions, we used a genetic fingerprint analysis to identify the genetic pattern of the donor and the recipient in the genomic DNA obtained from the cell populations isolated by cell sorting. The methodology employed was based on the amplification of 16 different STR regions that are highly polymorphic in the human genome. According to the amount of amplified material for each marker, in the case of mixed populations it is possible to estimate the percentage of material belonging to the different alleles. $CD3^+CD8^+$ T cells from patients 3, 5 and 6 were sorted at days 1, 2, 7 and 11 post-transplant. One hundred thousand $CD8^+$ T cells were sorted from a total of 1×10^7 mononuclear cells. A preoperative peripheral blood sample from the recipient and a blood sample from the donor were used as reference of individual fingerprint. We observed a progressive decrease in the number of $CD8^+$ donor T cells along the first post-operative week (Fig. 3). Dispersion in the donor/recipient ratio during the first 48 h post-ITx was due to early diminution of donor cells in patient 5. At later time-points, cells from the recipients become the only type of cells that can be recovered.

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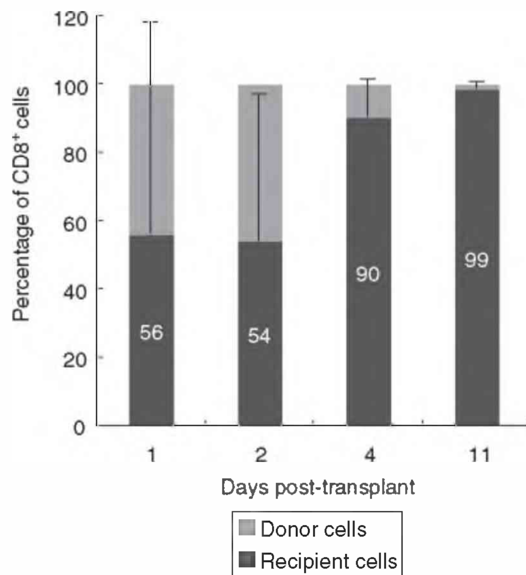

Fig. 3. Percentage of donor *versus* recipient CD8$^+$ T lymphocyte population in draining fluid along the early post-operative period. CD3$^+$CD8$^+$CD4$^-$ cells were sorted using the 'single cell' sort mode of BD FACSCalibur™ flow cytometer with sorting option. Purity was reassessed by fluorescence activated cell sorter (FACS) analysis in pilot experiments showing at least 95% of the desired population. Genomic DNA was prepared from CD8$^+$-sorted T cells and short tandem repeats (STR) were amplified by polymerase chain reaction (PCR) using a commercial kit. Allele discrimination in each case allows establishment of the ratio of donor/recipient cells in each sample. Mean and standard deviation from patients 3, 5 and 6 are depicted.

This result indicates that in the early post-operative period the recipient cell population replaces the circulating donor CD8$^+$ T cell population.

Discussion

Peritoneal fluid analysis has been accepted as a diagnostic tool to detect peritonitis, malignancies, portal hypertension, cardiac failure, tuberculosis, pancreatic or kidney disease [8]. There are guidelines for ascitic fluid analysis in cirrhotic patients with ascitis and bacterial peritonitis [9,10], but there are no specific guidelines for the analysis of abdominal fluid draining throughout the drains placed during surgery. Since the original descriptions of ITx, fluid collected from abdominal drainages has been used for clinical/surgical assessment, as has been performed for other organ transplants or general surgery, aiming to observe the presence of blood, bile, enteric or chylous content in the peritoneal cavity. The results presented here indicate that fluid collected from abdominal draining can also be used to analyse the presence, variations and graft/donor origin of immune cells migrating directly from the graft, opening a new dimension among the possibilities of post-ITx monitoring.

The process of restitution of the lymphatic network after ITx in humans has not been analysed. In animal models, it has been shown that lymphatic reconstitution begins within 2 weeks after ITx and is completed approximately 6 weeks after ITx [11,12]. In patients it is assumed that it might take a similar course, mainly by forming neo-lymphatic collaterals around the vascular anastomosis [13]. Along the immediate post-operative period, the daily volume of drained abdominal fluid decreases. This might be due partially to the neo-formation of lymphatic vessels. The amount of abdominal fluid recovered is dependent upon many aspects of patient care, such as fluid-electrolyte management, degree of dissection needed during the surgical procedure and introduction of enteral nutrition, among other factors. These aspects are reflected in the great dispersion of daily fluid volume obtained from one patient to another. Comparing the volume of draining fluid from 17 patients with isolated ITx and two patients who underwent combined liver-intestine Tx, the two combined Tx patients showed a seven-fold reduced volume of fluid. The lower volume collected in combined liver-intestinal transplant might be due to the lack of transection or dissection of the mesentery root, whereas the organ procurement for the isolated ITx the mesentery root is usually dissected (Fig. 1a). This observation underlines the finding that the intestinal lymphatic vessels leak into the peritoneal cavity, where the fluid is collected by Jackson–Pratt silicon drainage.

The lack of a correlation between the leucocyte population count in peripheral blood and the cellularity observed in the abdominal fluid (Fig. 1d) (usually much richer in lymphocytes) may indicate that the main cellular source of the draining fluid is disrupted lymph vessels that contain the outflow of leucocytes from the graft. Although information on cell populations present in human intestinal lymph is scarce, several animal studies have been performed to characterize different aspects of leucocytes migrating from intestinal mucosa. Most of the animal models are based on the cannulation of either afferent mesentery lymphatic vessels or the thoracic duct after surgical removal of mesenteric lymph nodes. These studies have shown that lymphocytes constitute the main population in the mesenteric lymph [14]. Furthermore, T lymphocytes are the predominant population, with a comparable amount of CD8$^+$ and CD4$^+$ cells, whereas B cells represent a minor component of the lymphoid populations [15–17]. Several studies have focused upon the characterization of dendritic cells migrating from the intestine [18,19], CD11b$^+$CD11c$^+$ CD103$^+$ being the major population [19,20]. Using FACS analysis we have observed an initial pattern with the presence of granulocytes, lymphocytes and monocytes during the first 3 days post-ITx. The strong presence of granulocytes might be associated with the immediate post-surgical inflammatory response in a procedure that involved opening of the intestinal lumen. In subsequent days, a shift towards a lymphocytic pattern was observed. Although we have analysed a restricted series of patients with different ages, immunosuppressive treatments and clinical outcomes, the CD4$^+$ or CD8$^+$ T cell counts were similar during the period analysed,

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being the major population retrieved, which is in agreement with the above-mentioned reports on animal models. When no clinical events were indicative of any inflammatory processes, we detected a decrease in both the B cell population and granulocytes during the post-operative period analysed. Conversely, monocytes and granulocytes increased markedly in the fluid of patients showing clinical events (data not shown). In certain cases, the changes in the cell composition of the draining fluid preceded the clinical events or changes in histology, indicating a potential use of this analysis as part of the clinical follow-up of the patient during the early post-transplant period.

We have also shown, by analysis of STR on DNA from sorted $CD8^+$ T cells, that it is possible to establish the relative contribution from donor or recipient to this population. We found that on post-operative day 2, 50% of the $CD8^+$ draining T cells already belong to the recipient, and by the 11th day almost all draining cells have been replaced by recipient cells. These results are in agreement with data reported by Wang *et al.* [21], who were using a mouse heterotopic ITx model showing that $CD4^+$ and $CD8^+$ donor T cells represent 50% of the total population in graft secondary lymphoid organs 1 day after Itx, and their proportion decreases to fewer than 2% at day 5 post-ITx.

Our results show for the first time the possibility of analysing lymphocyte trafficking from the graft in a human intestinal transplant. Further studies should be carried out to analyse whether the dynamic of replacement of other cell populations, such as $CD4^+$ T cells, $CD19^+$ B cells, NK cells, macrophages and dendritic cells takes place. The mean residence time of each population within the intestinal mucosa at this site may influence the dynamic of this trafficking, and also on the capacity to proliferate [22]. The discrimination of cell populations from the donor or recipient present in the abdominal fluid may allow estimation the half-life of different cell subsets within the intestinal mucosa, and also assessment of the kinetics of *de novo* colonization of different mononuclear lineages. Conversely, analysis of the donor–recipient origin of draining lymphocytes may have a direct impact in detection of the expansion of graft populations, leading potentially to graft-*versus*-host disease.

The first encounters of the recipient lymphoid cells with the donor cells take place during the early post-operative period [23]. As shown in an ITx animal model, priming of recipient T cells takes place mainly in graft secondary lymphoid organs early after transplant [21], suggesting that the monitoring activation markers on graft-draining lymphocytes could be a critical parameter to determine the risk of rejection.

Although not reported here, the fluid can also be used for evaluation of soluble factors, such as cytokines. Taking into account that mesenteric lymphatics are important contributors to the peritoneal cavity drainage, several determinations could be envisaged to monitor different important aspects of ITx such as bacterial translocation, enteral drug absorption or enteral metabolite profile. Furthermore, with a sufficient number of cases analysed, it would be possible to set up analytical parameters for expected normal evolution, and this information could be used for clinical follow-up and management.

In summary, we consider that the analysis of the abdominal draining fluid after intestinal transplant provides an innovative approach that can generate valuable information on the immunobiology of the intestinal graft, and could potentially become a useful tool in the patient care. Draining fluid analysis offers a unique opportunity to monitor the graft status and a direct method to analyse the biology of immune cells migrating outside the graft, improving our understanding on immune processes that take place in the early post-transplant period. Further analyses are necessary to define cellular patterns that could be associated with early rejection events or infections.

Disclosure

None of the authors have any conflict of interest with the subject matter or materials discussed in the manuscript.

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